Comparison of mass transfer models for determination of the intestinal permeability

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ABSTRACT

Background and the purpose of the study: In determination of the permeability of the intestinal wall by external perfusion techniques, several models have been proposed. In the present study three models were used for experimental results that differ in their convection and diffusion assumptions.

Material and Methods: Permeability coefficients for 13 compounds (metoprolol, propranolol, naproxen, ketoprofen, furosemide, hydrochlorothiazide, cimetidine, ranitidine, atenolol, piroxicam, antipyrine, ibuprofen and carbamazepine) with known human intestinal permeability values were determined in anaesthetized rats by different mass transfer models and plotted versus the observed human intestinal permeabilities.

Results: The calculated dimensionless wall permeability values were in the range of 0.37 – 4.85, 0.38-6.54 and 0.41-16.59 for complete radial mixing, mixing tank and laminar flow models respectively. The results indicated that all of the models work relatively well for our data despite fundamentally different assumptions. The wall permeabilities were in the order laminar flow > mixing tank > complete radial mixing.

Conclusion: Although laminar flow model provides the most direct measure of the intrinsic wall permeability, it has limitations for highly permeable drugs such as ibuprofen. The normal physiological hydrodynamics is more complex and more investigation is required to find out the real hydrodynamics.

Key words: Mass transfer, Permeability, Laminar flow, Perfusion, Mixing tank model

INTRODUCTION

Interest has grown in using *in vitro* and *in situ* methods to predict *in vivo* absorption potential of a drug as early as possible, to determine the mechanism and rate of transport across the intestinal mucosa and to alert the formulator about the possible windows of absorption and other potential restrictions to the formulation approach. Single-pass intestinal perfusion (SPIP) model is one of the mostly used techniques employed in the study of intestinal absorption of compounds which may be useful in prediction of absorbed oral dose and intestinal permeability in human. In determination of the permeability of the intestinal wall by external perfusion techniques, several models have been proposed (1-4). In each model, assumptions must be made regarding the convection and diffusion conditions in the experimental system which affects the interpretation of the resulting permeabilities. In addition, the appropriateness of the assumptions in the models to the actual experimental situation must be determined. Mixing tank (MT) model or well mixed model has been previously used to describe the hydrodynamics within the human perfused jejunal segment based on a residence time distribution (5). This model has also been used in vitro to simulate gastrointestinal absorption for assessment of the effects of drug and system parameters on drug absorption (6). However complete radial mixing (CRM) model was used to calculate the fraction dose absorbed and intestinal permeability of gabapentine in rats (7). Moreover these two models (MT and CRM) were utilized to develop a theoretical approach for estimation of fraction of the dose which is absorbed in human based on a macroscopic mass balance approach (MMBA) (8). Although these models have been theoretically explained, their comparative suitability to use for experimental data has not yet been reported. A comparison of proposed models will help to select the best model

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to establish a strong correlation between rat and human intestinal drug absorption potential. In this paper by using our data three common models for mass transfer in single pass perfusion experiments (SPIP) will be compared. The resulting permeability values differ in each model, which is interpretation rests on the validity of the assumptions. Thirteen compounds with known human intestinal permeability values enrolled in the study. There are only a limited number of drugs which their intestinal permeation in human volunteers have been investigated. Since human data are required for establishment of correlations, therefore tested drugs were selected from the list of those with known human effective intestinal permeabilities.

MATERIALS AND METHODS

Materials

Acetonitrile, Methanol, KH_2PO_4 , NaH_2PO_4, Na_2HPO_4, Orthophosphoric acid, NaOH, NaCl, Glacial acetic acid, Triethylamine, KCl and Sodium pentobarbital were from Merck (Darmstadt, Germany). Piroxicam, Ibuprofen, Carbamazepine, Naproxen, Phenol red and Ketoprofen were from Sigma (St. Louis, MO, USA). Furosemide and Hydrochlorothiazide were purchased from Shasun Chemicals and Drugs LTD (Pondicherry, India). Other materials which were used are as follows: Propranolol (ICI-Pharma, Madrid, Spain), Metoprolol (Ciba-Geigy) (Barcelona, Spain), Antipyrine (Across Belgum), Atenolol and Ranitidine (Uquifa, Spain), Cimetidine (Trifarma, Italy) were used in this investigation. Double-distilled water was used during the entire HPLC procedure.

Drug sample preparation

The tested drugs and their concentrations in PBS which were used as perfusion solution in SPIP studies are as follows: metoprolol (0.07 mM), propranolol (0.135 mM), naproxen (0.99 mM), ketoprofen (0.19 mM), furosemide (0.12 mM), hydrochlorothiazide (0.16 mM), cimetidine (0.39 mM), ranitidine (0.31 mM), atenolol (0.37 mM), piroxicam (0.03 mM), antipyrine (1.06 mM), ibuprofen (1.93 mM) and carbamazepine (0.42 mM). The pH of prepared buffer was adjusted to 7.2.

Assay of compound

All samples were analyzed by reverse-phase high performance liquid chromatography using Shimpack VP-ODS 5 µm 4.6 x 250 mm (Shimadzu, Kyoto, Japan) with a Shimpack VP-ODS 5 µm 4.6 x 50 mm guard column (Shimadzu, Kyoto, Japan). The detailed analytical data for tested drugs are as follows [9-12]:

For samples containing naproxen and ketoprofen the mobile phase was a mixture of methanol 19.9 % (v/v), acetonitrile 27.9 % (v/v), water 51.8 % (v/v) and triethylamine 0.4 % (v/v) (adjusted to pH 3.2). Metoprolol and propranolol were analyzed using methanol 55% (v/v), 0.05 M KH_2PO_4 45% (v/v) aqueous solution and triethylamine 0.2 % v/v (adjusted to pH 6) as mobile phase. Detection wavelengths were 270, 280 and 227 nm respectively. The mobile phase for furosemide, antipyrine and hydro-chlorothiazide samples consisted of acetonitrile 42% (v/v), water 58% (v/v), glacial acetic acid 0.9 % (v/v) and triethylamine 0.1% (v/v) (adjusted to pH 5.6). For other drugs the composition of mobile phases and detection wavelengths were as follows: piroxicam: acetonitrile 39 % (v/v), 0.1 M sodium acetate 61% (v/v) and triethylamine 0.05% (v/v) (adjusted to pH 2.6) λ=330 nm, carbamazepine: methanol 67% (v/v), water 33% (v/v) and glacial acetic acid 1% (v/v) λ=230 nm, atenolol: acetonitrile 10% (v/v), 0.67 M phosphate buffer adjusted to pH=7.4, 90% (v/v) and triethylamine 0.2% (v/v) (adjusted to pH 3) λ=225 nm, cimetidine & ranitidine: 0.05 M KH_2PO_4 78% (v/v), acetonitrile 22% and triethylamine 0.05% (v/v) (adjusted to pH 8) λ=229 nm, ibuprofen: acetonitrile 85% (v/v), 0.067 M phosphate buffer 15% (v/v) and Orthophosphoric acid 0.2% (v/v) λ=254 nm, phenol red: 0.05 M KH_2PO_4 45% (v/v) and methanol 55% (v/v) (adjusted to pH 2.6) λ=430 nm. The Reference Standards (RS) of compounds were used to quantitate the samples.

In situ intestinal absorption

In situ permeation studies were performed using established methods adapted from the literature [13]. A single pass constant flow (2 ml/min) of the drug containing perfusate (PBS pH=7.2, 37^{o}C) was established through the ligated rat intestine and the outlet samples were collected every 10 min in microtubes up to 90 min and stored at -20 ^{o}C until analysis. Finally the animal was euthanized with a cardiac injection of saturated solution of KCl. In all animal studies "Guide to the care and use of experimental animals" by Canadian Council on Animal Care, was followed [14].

RESULTS AND DISCUSSION

Mass transfer models

Three models are described that differ in their convection and diffusion assumptions (Fig 1). These models were the laminar flow, complete radial mixing (diffusion layer) for convective mass transport in a tube and the perfect mixing tank model. It is convenient to begin with the

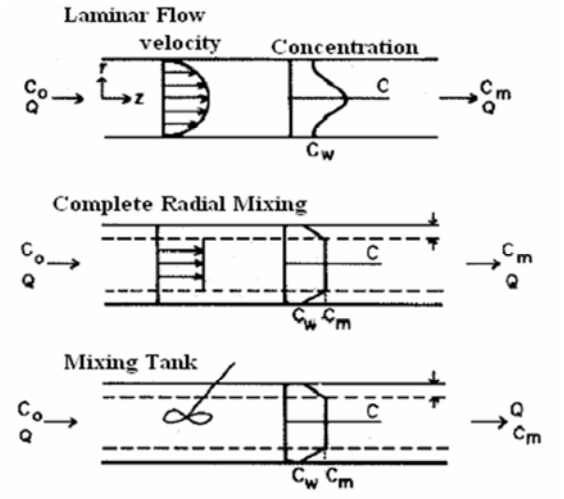

Figure 1. Velocity and concentration profiles for the models. The concentration profiles are also a function of z except for mixing tank model (4).

Table 1. Coefficients, $^{\circ}M_n$ and exponents, $^{\circ}\beta_n$ for the Graetz solution, equation (13), (sink conditions) (15)

(n)	$^{\circ}M_n$	$^{\circ}\beta_n$
1	0.81905	2.7043
2	0.09752	6.6790
3	0.03250	10.6734
4	0.01544	14.6711
5	0.00878	18.6699

solute transport equation in cylindrical co-ordinates [8,15,16]:

$$\nu^*_z \frac{\partial C}{\partial z^*} = Gz\,(\frac{1}{r^*}\frac{\partial}{\partial r^*} r^* \frac{\partial C}{\partial r^*}) \qquad \text{(Eq.1)}$$

$Z^* = Z / L$
$r^* = r / R$
$\upsilon^*_z = \nu_z / V_m$
$Gz = \pi DL/2Q$
R = radius of the tube
L = length of the tube
V_m = maximum velocity
Q = perfusion flow rate

This relationship is subject to the first-order boundary condition at the wall:

$$\left.\frac{\partial C}{\partial r^*}\right|_{r^*=1} = -P^*_w C_w \qquad \text{(Eq. 2)}$$

where P^*_w = P_w R/D = the dimensionless wall permeability.

The main assumptions achieving Eqs. 1 are: *(a)* the diffusivity and density are constant; *(b)* the solution is dilute so that the solvent convection is unperturbed by the solute; *(c)* the system is at steady state ($\partial C/\partial t = 0$); *(d)* the solvent flows only in the axial *(z)* direction; *(e)* the tube radius, R, is independent of Gz*;* and *(f)* axial diffusion is small compared to axial convection [16]. The boundary condition (Eq. 2) is true for many models having a tube wall but does not describe a carrier transport of Michaelis-Menten process at the wall, except at low solute concentrations.

Complete Radial Mixing mode:
For this model the velocity profile as with the plug flow model is assumed to be constant. In addition, the concentration is assumed to be constant radially but not axially. That is, there is complete radial but not axial, mixing to give, uniform radial velocity and concentration profiles. With these assumptions, the solution is written as:

$$C_m/C_0 = \exp(-4 P^*_{eff}\ Gz) \qquad \text{(Eq. 3)}$$

where P^*_{eff} replaces P^*_w [1-3]. Since no aqueous resistance is inc1uded in the model directly, the wall resistance is usually augmented with a film or diffusion layer resistance. That is, complete radial mixing occurs up to a thin region or film adjacent to the membrane. In this model the aqueous (luminal) resistance is confined to this region. Hence, the wall permeability includes an aqueous or luminal resistance term and can be written as:

$$P^*_{eff} = \frac{P^*_w P^*_a}{P^*_w + P^*_a} \qquad \text{(Eq. 4)}$$

where P^*_w is the true wall permeability and P^*_a, is the effective aqueous permeability. The aqueous permeability is often written as:

$$P_a = D/\delta \qquad \text{(Eq. 5)}$$

or

$$P^*_a = R/\delta \qquad \text{(Eq. 6)}$$

where δ is the film thickness and represents an additional parameter that needs to be determined from the data for determination of P^*_w. For typical experiments, P^*_a or R/δ is an empirical parameter, since the assumed hydrodynamic conditions may not be realistic at the low Reynolds numbers. The complete radial mixing model also can be derived from a differential

Table 2. The dimensionless permeability values determined based on three mass transfer models

Compound	Diffusivity [a] (*10^{-6}) cm^2/sec	Rat no.	Graetz no.	Mean P^*_{eff} (± SD) (CRM)	Mean P^*_{eff} (± SD) (MT)	Mean P^*_{wall} (± SD) (LF)
Atenolol	7.70	1	3.53E-02	0.37±0.00	0.38±0.00	0.41± 0.00
		2	3.46E-02			
		3	2.59E-02			
Cimetidine	8.70	1	3.32E-02	0.99±0.02	1.06±0.03	1.46± 0.07
		2	4.68E-02			
		3	3.98E-02			
Ranitidine	7.40	1	2.99E-02	0.55±0.02	0.57±0.25	0.67± 0.32
		2	3.16E-02			
		3	2.16E-02			
Antipyrine	9.92	1	5.34E-02	1.07±0.04	1.18±0.06	1.65± 0.13
		2	3.56E-02			
		3	4.45E-02			
Metoprolol	4.98	1	2.01E-02	1.21± 0.56	1.28±0.62	1.94± 1.35
		2	1.39E-02			
		3	1.68E-02			
Piroxicam	7.92	1	2.84E-02	1.80±0.92	2.09±1.18	11.70 ± 14.4
		2	3.56E-02			
		3	2.74E-02			
		4	3.48E-02			
		5	3.98E-02			
Propranolol	7.70	1	3.46E-02	1.32±0.48	1.50±0.61	2.72± 1.8
		2	3.98E-02			
		3	5.19E-02			
Carbamazepine	8.70	1	3.71E-02	1.29±0.12	1.42±0.14	2.17± 0.35
		2	3.94E-02			
		3	3.47E-02			
Furosemide	8.22	1	2.92E-02	0.72±0.44	0.76±0.47	0.98± 0.69
		2	2.36E-02			
		3	2.58E-02			
		4	3.87E-02			
Hydrochloro-thiazide	9.26	1	4.07E-02	0.39±0.21	0.41±0.22	0.46± 0.26
		2	4.24E-02			
		3	3.82E-02			
		4	4.15E-02			
Ibuprofen	7.40	1	3.82E-02	4.85±0.54	6.54±0.53	-------
		2	2.49E-02			
		3	2.76E-02			
Ketoprofen	8.42	1	3.40E-02	2.06±0.40	2.38±0.52	7.07±3.97
		2	3.02E-02			
		3	4.53E-02			
		4	2.72E-02			
Naproxen	8.55	1	3.26E-02	2.43±0.41	2.85±0.55	16.59± 15.8
		2	3.26E-02			
		3	2.92E-02			
		4	2.96E-02			

[a] Diffusivities were calculated using 2D structure of compounds applying the method proposed by Heyduk et al [17].

mass balance approach (1) and is often referred to the diffusion layer model. The Calculated P^*_{eff} values for the tested drugs and the corresponding plot are shown in table 2 and fig. 2 respectively.

Laminar flow model

For flow of a newtonian fluid in a cylindrical tube, the exit concentration of a solute with a wall permeability of P_w is given by [4,14]:

$$C_m/C_o = \sum_{n=1}^{\infty} M_n \exp(-\beta_n^2 Gz) \qquad \text{(Eq. 7)}$$

Where:

C_m = "cup-mixing" outlet solute concentration from the perfused length of intestine,
C_o = inlet solute concentration;

$$Gz = \pi DL/2Q \qquad \text{(Eq. 8)}$$

Gz is Graetz number, the ratio of the mean tube residence time to the time required for radial diffusional equilibration.
D = solute diffusivity in the perfusing fluid
L = length of the perfused section of intestine
Q = volumetric flow rate of perfusate = $\pi R^2(\upsilon)$
R = radius of perfused intestine
(υ) = mean flow velocity

Both the M_n and β_n in Eq. 7 are functions of P^*_w ,

the dimensionless wall permeability,

$$P_w^* = \frac{P_w R}{D} \qquad \text{(Eq. 9)}$$

From the form of the solution it appears that Gz is the only independent variable and that the solution is an implicit function of P_w^*. Since P_w^* (or P_w) is the parameter of interest, Eq. 4 is not in a convenient form for its determination, the following equations are defined.

:

$$\frac{1}{P_{eff}^*} = \frac{1}{P_w^*} + \frac{1}{P_{aq}^*} = \frac{1}{{}^{\circ}P_w^*} + \frac{1}{{}^{\circ}P_{aq}^*} \qquad \text{(Eq. 10)}$$

$$P_{eff}^* = \frac{\ln[(C_m/C_0)]_{exp}}{-4Gz} \qquad \text{(Eq. 11)}$$

$${}^{\circ}P_{aq}^* = \frac{\ln[(C_m/C_0)]_0}{-4Gz} \qquad \text{(Eq. 12)}$$

$$[(C_m/C_0)]_0 = \sum_{n=1}^{5} {}^{o}M_n \exp(-{}^{\circ}\beta_n^2\, Gz) \qquad \text{(Eq. 13)}$$

where the superscript ^{o} denotes the sink condition (Graetz solution), the superscript * denotes dimensionless quantities [Eq. 8] and subscripts exp stands for experimental condition. The wall permeability is determined in the following manner: First the ${}^{\circ}P_{aq}^*$ is calculated using Eqs. 8 , 10, 13 and table 1. Then the P_{eff}^* is calculated from the experimental results using Eq. 8 and 11 at the third step the value of ${}^{\circ}P_w^*$ is found out from Eq. 10 and finally the value of ${}^{\circ}P_w^*$ is multiplied by the correction factor in fig 3 to obtain P_w^*. All calculations were performed in SPIP model. The Gz values were calculated based on equation 8, using the compound diffusivity, length of intestine and flow rate of perfusion which are shown in table 2. The average value of Gz was found to be 3.34×10^{-2} ($\pm$ 8.6×10^{-3}). It seems that there are limitations for the use of laminar flow model in determination of the dimensionless wall permeability of highly permeable drugs. For instance a negative value of ibuprofen dimensionless wall permeability was obtained based on laminar flow model because of the high P^*_{eff} value of ibuprofen in comparison with its calculated P^*_{aq} sink value and as a result the drug was excluded from correlation plot. Table 2 also represents the obtained dimensionless rat gut wall permeabilities (P_w^*) for the tested compounds. The plot of P_w^* versus the observed human intestinal permeability values is shown in fig. 3.

Mixing Tank Model

This model takes the next step and assumes that both radial and axial mixing are complete. The aqueous resistance is again believed to be confined to a region (film) next to the membrane where only molecular diffusion occurs, and the rest of the contents are well mixed (perfect mixer). This model is described most easily by a mass balance on the system:

$(\text{mass/time})_{inlet}$-$(\text{mass/time})_{outlet}$= $(\text{mass/time})_{absorbed}$

or:

$$QC_0 - QC_m = (2\pi RL)(P'_{eff})C_m \qquad \text{(Eq. 14)}$$

where $2\pi RL$ is the area of the mass transfer surface (cylinder) of length *L* and radius *R*, P'_{eff} is the permeabilily or mass transfer coefficient of the surface, and C_m is the concentration in the tube (which is constant and equal to the outlet concentration by the perfect mixing assumption). From Eq. 14 it is obtained:

$$\frac{C_0 - C_m}{C_m} = P'_{eff}\frac{2\pi RL}{Q} \qquad \text{(Eq. 15)}$$

or:

$$C_0/C_m = 1 + 4P'^{*}_{eff}Gz \qquad \text{(Eq. 16)}$$

As with the complete radial mixing model, P^*_{eff} contains additional parameter $P'^{*}_a = R/\delta'$ that must be estimated from the data, the P'^{*}_a and P'^{*}_{eff} values for the mixing tank model differ from those for the complete radial mixing model by nature of the different hydrodynamic assumptions [4]. While this model is not appropriate to most perfusion experiments, it is useful to compare its ability for correlation of mass transfer data with other models. However P^*_{eff} for our data was calculated on the basis of assumptions of mixing tank model. The data and representative plots for this model are shown in table 2 and fig. 5 respectively.

The calculated dimensionless wall permeability values were in the range of 0.37 – 4.85, 0.38-6.54 and 0.41-16.59 for complete radial mixing, mixing tank and laminar flow models respectively. It is clear that drugs with different physicochemical properties of all four

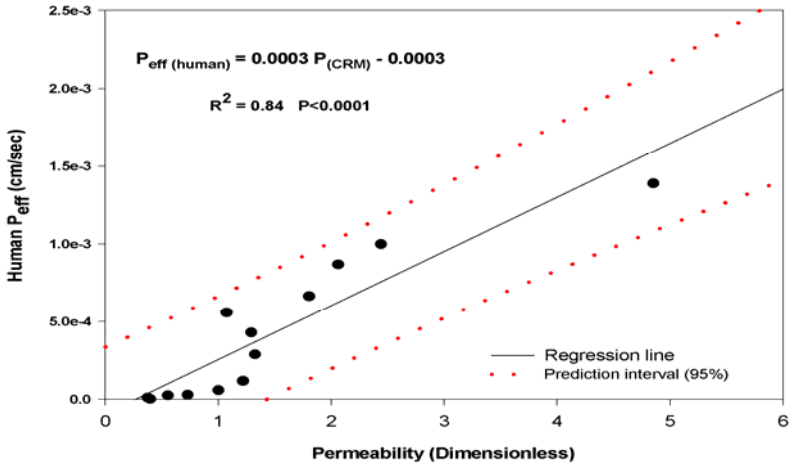

Figure 2. Plot of dimensionless permeability values vs human P_{eff} values in complete radial mixing model.

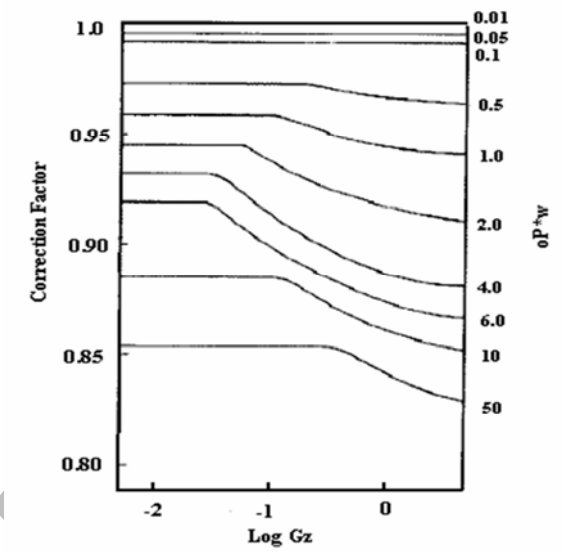

Figure 3. Correction factor to obtain exact wall permeability (P_w^*) given the estimated wall Permeability ($^{\circ}P_w^*$) and value of *Gz*. (15).

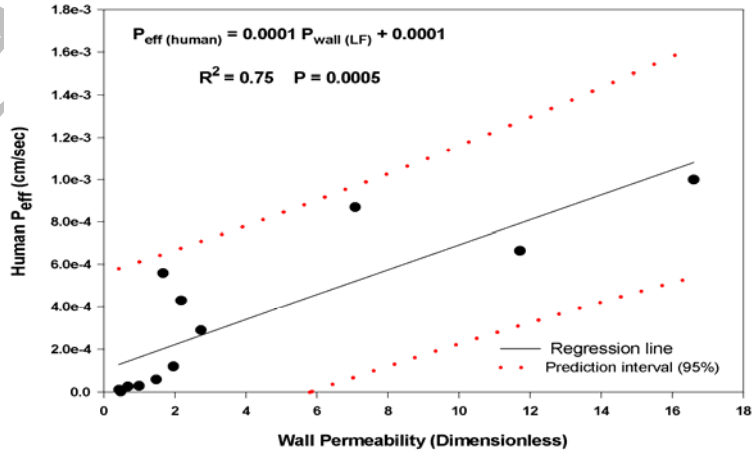

Figure 4. Plot of dimensionless rat gut wall permeability values vs human P_{eff} values in laminar flow model

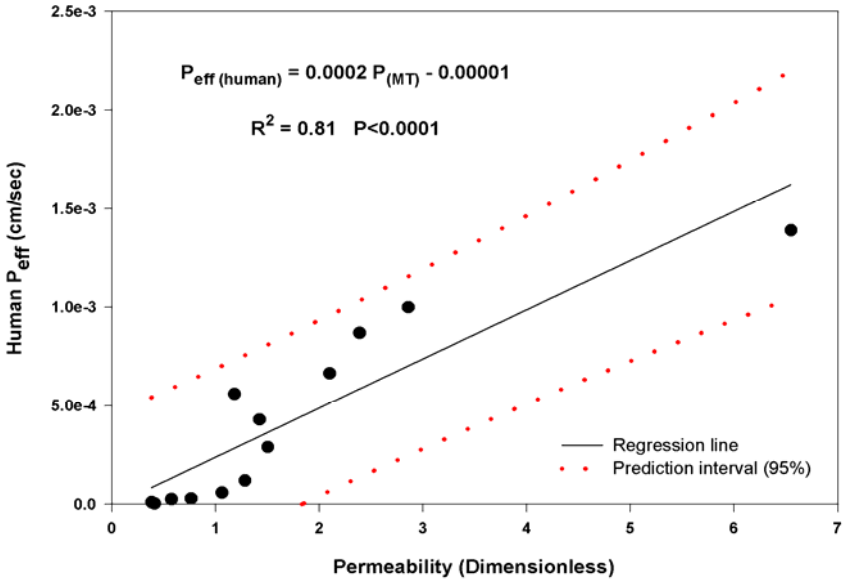

Figure 5. Plot of dimensionless permeability values vs human P_{eff} values in mixing tank model

biopharmaceutical classes were enrolled in the study. Atenolol a class III drug (high soluble-low permeable) showed lowest effective permeability value in all three investigated models. It is also shown that there is only a small difference in the calculated atenolol permeability coefficients in three models. However this variation becomes more salient for high permeable drugs; i.e. class I (high soluble-high permeable) and class II (low soluble-high permeable) drugs especially in term of permeability in laminar flow model. For instance the observed mean permeability values for naproxen, a class II drug, are 2.43, 2.85 and 16.59 in CRM, MT and LF models respectively. Therefore it seems that in comparison to other model laminar flow model provides larger values for highly permeable drugs in comparison to the other models. However the ranking order for intestinal absorption of tested drugs is almost the same in other evaluated models. In addition it seems that it would be possible to classify drugs correctly by the resulting values.

Fig. 2, 4 and 5 demonstrate the obtained correlations for investigated models. It is seen that the plots of rat permeability versus human P_{eff} values, present rather high linear correlations with intercepts not markedly different from zero (R^2= 0.81, P <0.0001 for MT, R^2= 0.75, P =0.0005 for LF, R^2= 0.84, P <0.0001 for CRM). The permeability of various models is different. The permeabilities resulting from application of the other models can be interpreted if it is assumed that the laminar flow permeability measures the wall permeability. The permeability values for the complete radial mixing model are lower than the laminar flow model since this model assumes radial mixing, which leads to lower estimated luminal (aqueous) resistance values and a higher estimated membrane resistance (lower permeability value). However, the usual interpretation of the complete radial mixing model recognizes that the permeability value includes an aqueous resistance. While the permeabilities in mixing tank model, which takes the final step in assuming both radial and axial mixing, were expected to be the lowest among all models, they were in the range between permeabilities in complete radial mixing and dimensionless wall permeabilities. Although theoretically laminar flow model has been established as a reasonable approximation in external perfusion studies, based on the results of correlations of this study, it seems the hydrodynamics in normal physiological situation clearly are more complex and require further investigation to choose from proposed models.

CONCLUSION

All investigated models work relatively well for our data despite fundamentally different assumptions. The wall permeabilities fall in the order laminar flow > mixing tank > complete radial mixing. Based on the resulting correlations it may be concluded that although laminar flow model provides the most direct measure of the intrinsic wall permeability, it has limitations for highly permeable drugs such as ibuprofen and the normal physiological hydrodynamics is more complex and further investigations are required to find real hydrodynamics.

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