

Tutorial



Predicting human pharmacokinetics from preclinical data: absorption

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ABSTRACT

Predicting the rate and extent of oral absorption of drugs in humans has been a challenging task for new drug researchers. This tutorial reviews *in vitro* and PBPK methods reported in the past decades that are widely applied to predicting oral absorption in humans. The physicochemical property and permeability (typically obtained using Caco-2 system) data is the first necessity to predict the extent of absorption from the gut lumen to the intestinal epithelium (F_a). Intrinsic clearance measured using the human microsome or hepatocytes is also needed to predict the gut (F_g) and hepatic (F_h) bioavailability. However, there are many issues with the correction of the inter-laboratory variability, hepatic cell membrane permeability, CYP3A4 dependency, etc. The bioavailability is finally calculated as $F = F_a \times F_g \times F_h$. Although the rate of absorption differs by micro-environments and locations in the intestine, it may be simply represented by k_a . The k_a , the first-order absorption rate constant, is predicted from *in vitro* and *in vivo* data. However, human PK-predicting software based on these PBPK theories should be carefully used because there are many assumptions and variances. They include differences in laboratory methods, inter-laboratory variances, and theories behind the methods. Thus, the user's knowledge and experiences in PBPK and *in vitro* methods are necessary for proper human PK prediction.

Keywords: Absorption; Bioavailability; PBPK; Human PK Prediction

INTRODUCTION

Predicting human pharmacokinetics (PK) of candidate molecules in preclinical development has been an essential task for the successful planning of forthcoming clinical development. Appropriate human dosage regimens are estimated based on predicted human PK exposure, and the human exposure is calculated using four PK parameters of bioavailability (F), oral absorption rate constant (k_a), the volume of distribution (V_d), and clearance (CL). We are planning to publish a series of tutorials on the method of human PK prediction in TCP for education. PIPET (Pharmacometrics Institute of Practical Education and Training) of the Catholic University of Korea has distributed a freely-available human PK-predicting software system named Dallphin-AtoM (ver. 0.9.1 is currently available). After a thorough review of published reports on PBPK or allometric methods to predict human PK parameters, we

Author Contributions

Conceptualization: Yim DS, Choi S, Bae SH;
Data curation: Choi S; Funding acquisition: Yim DS;
DS; Methodology: Choi S, Bae SH; Supervision: Yim DS;
Writing - original draft: Yim DS;
Writing - review & editing: Yim DS, Choi S, Bae SH.

GLOSSARY

- A** calculated human small intestinal cylindrical surface area (0.66 m²)
- CL_{int}** intrinsic clearance
- CL_{int,app}** the overall hepatic intrinsic clearance
- CL_{int,met}** microsomal metabolic intrinsic clearance
- CL_{int,pass}** intrinsic passive diffusion clearance across the hepatocyte cell membrane
- CL_{int,gut}** intrinsic metabolic clearance in the gut
- CL_{int,gut}** hepatic clearance
- CL_{perm}** permeability clearance through the enterocytes
- F_a** the fraction absorbed from the gut lumen to enterocytes
- F_g** the fraction escaping first-pass gut wall metabolism
- F_h** the fraction escaping hepatic first-pass metabolism
- k_a** the absorption rate constant
- P_{app}** apparent permeability
- P_{eff}** effective permeability, human jejunal permeability
- Q_{ent}** the average enterocytic blood flow (18 L/h)
- Q_{gut}** a hybrid parameter reflecting both the villous blood flow and permeability to the enterocyte membrane
- R** radius of the human small intestine (2 cm)
- S** intestinal surface area (200 m²)
- T_{res}** transit time in the human small intestine (3 h)
- V_c** central volume of distribution

incorporated selected methods into Dallphin-AtoM. Thus, we expect that these tutorial series help readers gain insights on how the methods are applied in real fields and gain knowledge on the logic and methods of human PK prediction.

PREDICTION OF ORAL ABSORPTION

Oral absorption of drugs is represented by two parameters, bioavailability (F, the extent of absorption) and absorption rate constant (k_a, parameter on the speed of absorption). The absorption lag time may also matter in humans, but not in animal (*in vivo*) PK because most of the studies are done using oral solutions. Likewise, *in vitro in vivo* correlation (IVIVC) approaches using dissolution test data of oral formulations are not discussed because such formulations are rarely prepared in early discovery or preclinical stages.

The loss of drugs in the oral absorption process occurs at three locations of the intestinal lumen, enterocytes, and hepatocytes, as expressed in the following equation.

$$F = F_a \cdot F_g \cdot F_h \tag{Eq. 1}$$

F_a is the fraction absorbed from the gut lumen to enterocytes, F_g is the fraction escaping first-pass gut wall metabolism, and F_h is the fraction escaping hepatic first-pass metabolism (**Fig. 1**).

THEORY AND METHODS TO PREDICT F_a

F_a predicted with Caco-2 permeability

In the intestinal lumen, F_a is determined by two factors; permeability and solubility. However, the solubility that governs the rate and extent of a formulation to be dissolved in the GI fluid is not used in F_a's prediction because the formulation of test drugs is always a solution or suspension at *in vitro* and *in vivo* (animal) studies. Permeability through the cell membrane

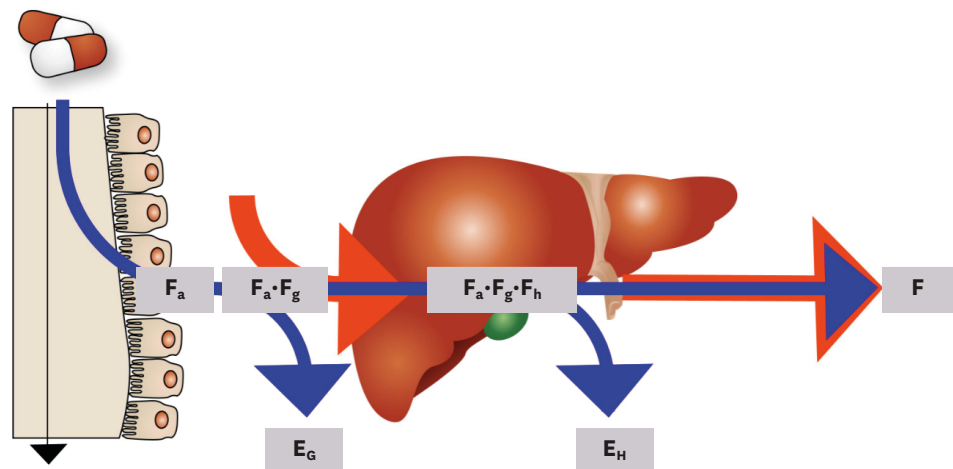


Figure 1. Three components of bioavailability (F). $F = F_a \times F_g \times F_h$. F_a, the fraction absorbed from the gut lumen to enterocytes; F_g, the fraction escaping first-pass gut wall metabolism; F_h, the fraction escaping hepatic first-pass metabolism.

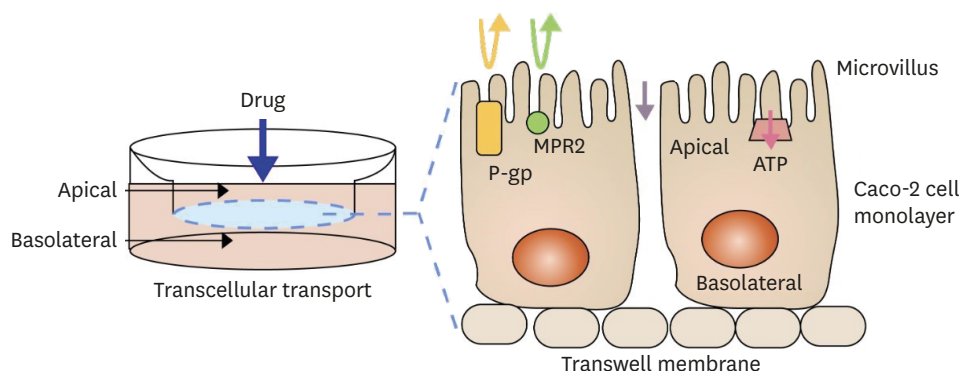


Figure 2. Method to measure membrane permeability of drugs using the Caco-2 cell system.

reflexes the electric charge, polarity, molecule size, carrier-mediated transportation of drugs (**Fig. 2**), and the permeability through the intestinal epithelium is what we need to predict.

Earlier attempts to measure intestinal permeability of drugs were rather direct. *Ex-vivo* methods using animal intestine or clinical studies with endoscopic balloons (see Box 1) to measure drug concentrations absorbed or remaining in the lumen are those, but they are too demanding. Thus, *in vitro* methods were developed to replace *ex vivo* or clinical permeability studies. The following two equations represent the logic of predicting F_a from *in vitro* permeability data.

$$F_a = 1 - e^{\left(\frac{-2P_{eff} \cdot T_{res}}{R}\right)} \tag{Eq. 2}$$

$$\log(P_{eff}) = 0.4926 \cdot \log(P_{app}) - 0.1454 \tag{Eq. 3}$$

P_{eff} (effective permeability, 10^{-4} cm/s) indicates human jejunal permeability measured by the endoscopic balloon method [1] in humans. As shown in the Eq. 2 and **Fig. 3**, P_{eff} and T_{res} (transit time in the human small intestine, 3 h) and R (radius of the human small intestine, 2 cm) predict F_a in humans [1].

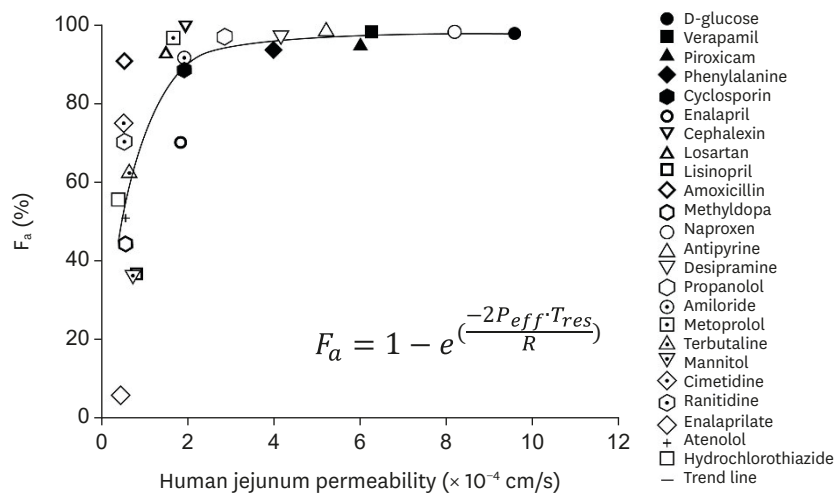


Figure 3. Correlation between human jejunal permeability (P_{eff}) and F_a in 24 drugs: redrawn from the report by Sun et al. [2]. The equation is from [1]. P_{eff} , effective permeability, human jejunal permeability.

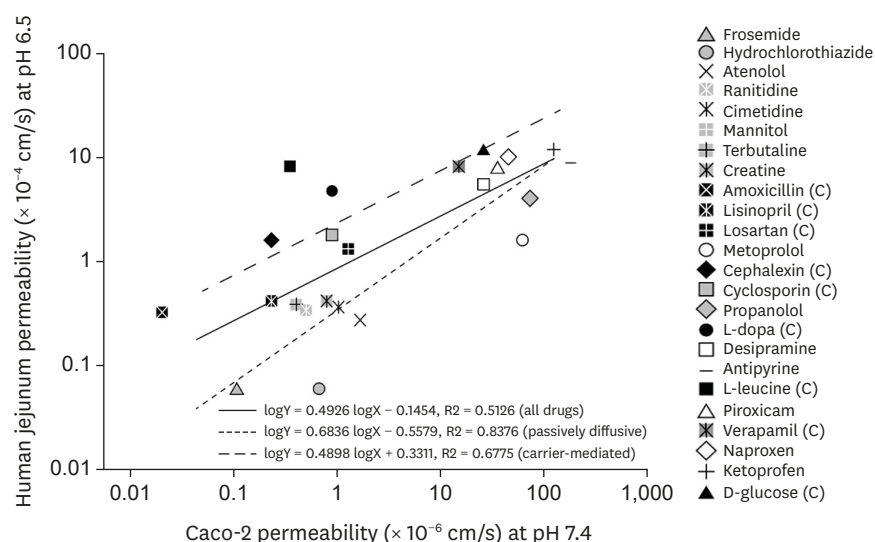


Figure 4. Correlation between Caco-2 permeability (P_{app}) and human jejunal permeability (P_{eff}) redrawn from the report by Sun et al. [2].

P_{app} , apparent permeability; P_{eff} , effective permeability, human jejunal permeability.

As mentioned above, P_{app} (apparent permeability, 10^{-6} cm/s) obtained from the Caco-2 permeability study is frequently used instead of P_{eff} because both are closely correlated [2], and the *in vitro* Caco-2 study is much simpler than the endoscopic balloon study to obtain the P_{eff} in humans. The correlation between P_{app} and P_{eff} is shown in **Fig. 4**. In summary, P_{app} from the Caco-2 study is used to estimate P_{eff} , the jejunal permeability (Eq. 3), and the estimated P_{eff} , thereby is incorporated into the equation to calculate F_a (Eq. 2).

Methods to measure *in vitro* permeability

When Caco-2 cells are used to measure *in vitro* permeability, P-gp substrates will have some merits in the Caco-2 system than using other methods because the P-gp transporter is expressed in the apical membrane of Caco-2 cells. When a drug is not a substrate of P-gp, using MDCK (Madin-Darby canine kidney) cells may also be an alternative to measure passive enterocyte membrane-permeability. Without regard to the Caco-2 or MDCK, the *in vitro* permeability measurement is prone to substantial inter-laboratory variation. Thus, P_{eff} of reference substances such as propranolol and atenolol are frequently measured as reference standards together with the test drug to minimize the inter-laboratory variation.

Caveats of F_a prediction using *in vitro* permeability

As demonstrated in **Fig. 3**, drugs with permeability (P_{eff}) lower than 1×10^{-4} cm/s show a wide range of F_a values compared with those with higher permeability. Currently, there is no other reliable method to predict F_a for these low permeability molecules. Thus when such molecules are investigated, the predicted F_a should be carefully interpreted.

F_a predicted with PSA

There is another approach to predict F_a . In a molecule's physicochemical properties, the PSA (polar surface area) is defined as the area occupied by nitrogen and oxygen atoms and hydrogen atoms attached to them. It was reported that a single physicochemical descriptor (e.g., lipophilicity) often fails to predict drug absorption because the diversity in the molecular structure is not adequately considered [3]. Meanwhile, a reversed sigmoid-form (inverse) correlation between PSA and F_a reported previously [4] may also be considered for

<Box 1>: How is F_a measured?

Because the absorption into enterocyte cannot be measured separately from the enterocyte or liver's metabolism, the direct measurement of F_a in the human body is not possible. Instead, investigators have used the endoscopic balloon method (**Fig. 5**) in the human jejunum to estimate the F_a together with P_{eff} (jejunal permeability) (5). Both estimated P_{eff} and F_a depend on drug concentration changes in the jejunal lumen, but in the case of F_a , fluid flow in the lumen is also used (not in P_{eff}). Thus, F_a is not determined by P_{eff} only, although they are closely correlated as in the Eq. 2.

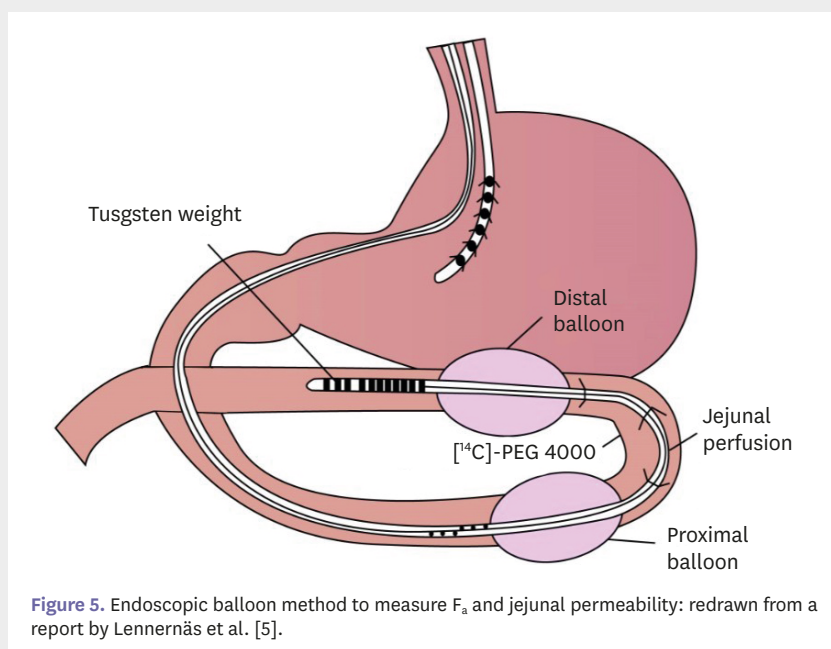


Figure 5. Endoscopic balloon method to measure F_a and jejunal permeability: redrawn from a report by Lennernäs et al. [5].

the prediction of F_a . We derived a least square equation for the correlation curve by harvesting the raw data [4] in their figure to apply at Dallphin-AtoM. However, when we tried to validate the correlation between the PSA and F_a by collecting published information (PSA and F_a data) of drugs other than those used in their report [4], the correlation was somewhat unclear (the comparison results yet unpublished). Moreover, the PSA method may predict passive permeability only, not the transporter-mediated permeability. Thus, we do not recommend the PSA as the first choice to predict F_a over Caco-2 permeability despite the published fancy correlation curve [4]. The PSA method may be considered if the Caco-2 permeability-derived F_a is not much reliable (e.g., when $P_{eff} < 1 \times 10^{-4}$ cm/s).

THEORY AND METHODS TO PREDICT F_g

Measurement of hepatic CL_{int} - an initial step for estimation of F_g

The intestinal cell is the first barrier that the drug contacts in the human body. The absence of plasma protein binding in the gut lumen and the long intestinal transit time (~4 hours) make the enterocytes a significant place for drug elimination before entering the liver.

Both of the F_g and F_h are determined by the CL_{int} by drug-metabolizing enzymes in the liver or intestine. In the case of F_g , the CYP3A4 is the known drug-metabolizing enzyme dominant in the intestinal epithelium (> 80% of the CYP enzymes in enterocytes), whereas all kinds of CYP and UGT enzymes contribute to the hepatic metabolism. For both of F_g and F_h , measuring hepatic CL_{int} is the first task to be done *in vitro*. Calculation of the F_g and F_h is initiated with obtaining the hepatic CL_{int} data. Although the intestinal CL_{int} may also be measured *in vitro*, it is still a costly method; $CL_{int,gut}$, the hepatic CL_{int} of the test drug scaled by its metabolic fraction by CYP3A4 and the total amount of CYP3A4 in enterocytes and hepatocytes, is frequently used instead of measuring the intestinal CL_{int} using intestinal microsomes to calculate the F_g . (Methods to determine the metabolic fraction by different CYP and UGT isozymes are not discussed in this tutorial.) You should remember that the $CL_{int,gut}$ is generally calculated using the microsome-measured CL_{int} , but not the intact hepatocyte-measured CL_{int} .

Drugs metabolized by UGT

The methods presented above are those for the drugs metabolized by CYP450 isozymes. However, some drugs are eliminated mainly by enzymes other than CYP in the liver, e.g., UGT. *In vitro* methods for these minority drugs are not discussed here, but the principle of calculating CL_{int} and F_h is identical. A few drugs whose major metabolic pathway is by UGT may be eliminated by the UGTs existing in enterocytes, but methods to calculate F_g for these drugs are not established yet.

The Q_{gut} model

Now, assume that we got the intestinal CL_{int} value by a direct *in vitro* study or the $CL_{int,gut}$ by correcting the hepatic CL_{int} with CYP3A4-mediated metabolic fraction and the total amount of intestinal CYP3A4 (70.5 nmol). What can we do with this to obtain the F_g ? Although there is no consensus in the method to estimate F_g , the Q_{gut} model proposed by Yang et al. [6] is rather frequently applied. The equations as following are used for this purpose.

$$CL_{perm} = P_{eff} \times A \quad \text{Eq. 4}$$

$$Q_{gut} = \frac{CL_{perm} * Q_{ent}}{CL_{perm} + Q_{ent}} \quad \text{Eq. 5}$$

$$F_g = \frac{Q_{gut}}{Q_{gut} + CL_{int,gut}} \quad \text{Eq. 6}$$

The Q_{gut} in Eq. 5 is a hybrid parameter reflecting both the villous blood flow and permeability to the enterocyte membrane. Q_{ent} is the average enterocyte blood flow (18 L/h). CL_{perm} in Eq. 4 represents the CL by permeation into the enterocyte calculated as the human small intestinal cylindrical surface area (A, 0.66 m²) times the P_{eff} , the human jejunal permeability. The F_g is predicted using CL_{perm} and Q_{gut} as in Eq. 6 [7]. The CL_{perm} in Eq. 4 may also be calculated using P_{app} (Caco-2 permeability) \times S (intestinal surface area amplified by the microvilli, 200 m²). However, the gap between the ($P_{eff} \times A$) and ($P_{app} \times S$) remarkably increases in high permeability drugs. Moreover, the CL_{perm} or F_g used in the equations above are conceptual terms and their actual measurement is not possible. Thus, there is no consensus on the choice between the two methods yet.

THEORY AND METHODS TO PREDICT F_h

Correction of microsome-measured CL_{int}

Hepatocytes or microsome?

When you use human hepatocytes instead of microsome to measure CL_{int} , this permeability correction step is not necessary because the drug's diffusion rate through the cell membrane is already incorporated in the method and results. However, all these logics do not always apply because of severe inter-laboratory, inter-occasion variability of *in vitro* metabolism studies, and the influences by uptake transporters. The investigator should consider many factors carefully when correcting the microsomal CL data because such correction may not guarantee improved accuracy in the estimated CL_{int} . Detailed methods to calculate hepatic CL_{int} is to be discussed in the upcoming tutorial on human CL prediction.

Permeability correction method for microsome-measured CL_{int}

Hepatic CL_{int} is frequently measured using human hepatocytes or microsome. Because there is no cell membrane (as a barrier to drugs) in the microsomal preparation, unlike hepatocytes, the measured metabolic rate may be substantially overestimated in drugs with low membrane permeability. To correct this discrepancy, we may apply passive diffusion CL to the microsome-measured initial values of intrinsic metabolic CL to reflect the diffusion rate of drug molecules across the hepatocyte cell membrane [8]. In the Eq.7, the method to correct the $CL_{int,met}$ with the $CL_{int,pass}$ is shown.

$$CL_{int,app} = \frac{CL_{int,met} * CL_{int,pass}}{(CL_{int,met} + CL_{int,pass})} \quad \text{Eq. 7}$$

$CL_{int,app}$, the overall hepatic intrinsic CL represents the corrected microsomal CL_{int} ($CL_{int,met}$). In the equation reported by Li et al. [8], the intrinsic CL by biliary excretion and uptake or efflux transporter influences were originally included. Because they are rarely measured, it was simplified like in the Eq. 7. The passive diffusion CL comes from the P_{app} obtained from the MDCK-II-LE (Madin-Darby canine kidney II-low efflux) cell monolayer permeability assay. ($CL_{int,pass} = P_{app} \times 2 \times SA_{HHEP}$). The SA_{HHEP} is the surface area of 1 million hepatocytes ($13.52 \mu\text{m} \times 10^6$ cells), and P_{app} is the permeability (units of 10^{-6} cm/s) measured using the MDCK-II cells. When the MDCK-II permeability data is not available, the user may also try the Caco-2 permeability data using a linear correlation between the two methods.

Whether to correct the microsomal data with the membrane permeability should be carefully decided by researchers. Huge inter or intra-laboratory variance involved in the *in vitro* experiments on microsomal CL and permeability may make the decision more challenging than what is simply reasoned from theories.

Three models to calculate F_h

In the case of F_h , three models using CL_{int} are so far known: well-stirred model, parallel tube model, and dispersion model. Although their performance in estimating F_h is similar for many drugs, there is a report that the well-stirred model enormously overestimates the F_h in a few drugs with F_h lower than 10%, and the dispersion model appeared better at this situation [9]. The dispersion model assumes the concentration gradient from the capillary inlet to the outlet as in the parallel tube model and free diffusion of solutes along the blood flow axis [10].

Likewise, there is no consensus in choosing one model over the others in predicting the CL_h , and the well-stirred model is still the most frequently used to predict CL_h . A detailed discussion will be done at the next tutorial for CL prediction. It should be remembered that both the Q_h and CL_h used in PBPK approaches, including F_g and F_h are those of the blood, not the plasma.

THEORY AND METHODS TO PREDICT THE ABSORPTION RATE

Sophisticated models

The traditional assumption on the first-order absorption may not apply when the saturable process by enzymes or transporters are involved. Because the absorption rates and metabolic rates differ by the segments of the intestine, attempts to predict F_g , F_a , and absorption rates using the information such as the physicochemical property of drugs, pH, CYP activity, transporter, blood flow in each segment resulted in the development of the ADAM model [11] or the ACAT model [12]. Although the concept was proposed long ago, the detailed parameter information at each intestinal segment is not completely open to the public. Methods to predict the F terms, F_a , F_g , and F_h using known physicochemical property and *in vitro* results were discussed in the previous sections, and they are used to predict human F without depending on those sophisticated models. To properly utilize the ADAM or ACAT models, we need detailed information on test drugs including the metabolic fraction by each CYP and UGT isozymes. To prepare all such data in the preclinical development stage is not realistic.

Practical method to predict k_a

In the case of the absorption speed, k_a , the first-order absorption rate constant, can be modeled using a couple of prediction methods. A method reported by Usansky et al. [13] showed a satisfactory prediction of reported human oral PK profiles (peak concentrations) in 18 drugs using the equation as follows.

$$k_a = \frac{P_{app} \cdot S}{V_c} \quad \text{Eq. 8}$$

P_{app} is the drug permeability across the intestinal mucosa (Caco-2 permeability), S is the absorptive surface area (200 m²), and V_c is the central volume of distribution. Methods to predict human V_c will be discussed in upcoming tutorials. Also, allometric approaches or simple averaging of k_a values obtained from PK of different animal species may be used when there is no appropriate data to predict k_a . Although the first-order absorption model has long been criticized as inappropriate to describe oral drug absorption, it is the most frequently used for its simplicity. Moreover, the oral formulation given in the clinical phases is never in solution or suspension forms: the formulation differences between animals (solution or suspension) and humans (capsule or tablet) tend to perturb the absorption rates predicted from preclinical data. The use of a sophisticated prediction model does not help this perturbation. Thus, using the simple first-order absorption model in the prediction of human PK is still worth trying.

IMPLEMENTATION IN DALLPHIN-ATOM

We have been developing a free software system, “Dallphin-AtoM” to predict human PK using *in vitro* and animal data since 2018 (http://pipet.or.kr/board/apps_list.asp). The name is the acronym of DRugs with ALLometry and PHysiology INSide – Animal to huMan. Our initial

DallPhin-AtoM (0.9.1 ver)

File Edit Help

Basic info Absorption Distribution Elimination **Final Parameters** Conc. Prediction Full PBPK About

Prediction in a 70-kg human

CL : *error*

F : *0.0*

Q : *output*

Vss : *output*

Vc : *output*

Ka : *0.0 /hr*

Fh(Microsome)

Fh(CLh) *1.0*

Fh(CLh_fromRativivc) *NaN*

Fh(CLh_PS) *NaN*

Fh(CLh_PS(logD)) *1.0*

User input

Fh*Fg*Fa *1.0*

Fg

Fg *1.0*

Fg(HLM) *0.0*

Fg(HIM) *0.0*

Fa

Fa_Caco *0.0*

Fa_PSA *1.0*

Figure 6. Final parameter tab in the Dallphin-AtoM ver. 0.9.1.

goal was to develop free software that enables users to apply PBPK methods (physicochemical data and *in vitro* experimental data) and allometric scaling approaches as they like. Moreover, we aimed to let users clearly understand how the output presented by the software were calculated. All the methods reviewed in this manuscript were implemented in Dallphin-AtoM except for the complicated absorption models (ACAT and ADAM). After filling up the Basic info, Absorption, and Elimination tabs with appropriate input data, the user obtains F and k_a in the Final parameters tab. Depending on the kinds of input data, the user may obtain as many as four F_h , two F_g , two F_a values, and one k_a (Fig. 6) in its 0.9.1 version. However, which one to pick between these multiple candidate parameters is the users' responsibility, and their knowledge and experiences are critical to getting the most from any human PK-predicting software.

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