

Review

Intestinal First-Pass Metabolism of CYP3A4 Substrates

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Summary: Cytochrome P450 3A4 (CYP3A4) is present not only in the liver but also in the small intestine, where it functions as a barrier against xenobiotics. Some CYP3A4 substrates exhibit low bioavailability due to intestinal first pass metabolism. The AUCs of such CYP3A4 substrates are remarkably changed by the inhibition, induction, and saturation of CYP3A4 and so prediction of intestinal first-pass metabolism is important. In this article, factors affecting intestinal first-pass metabolism of drugs are reviewed, focusing on the intestinal metabolism by CYP3A. The methods to predict intestinal first-pass metabolism are also reviewed.

Keywords: intestinal metabolism; CYP3A4; prediction; grapefruit juice

Introduction

When given orally, a drug is absorbed from the gut to systemic circulation via the liver. The fraction of the administered drug entering the blood circulation is referred to as bioavailability (F) and is expressed by the equation:

$$F = Fa \cdot Fg \cdot Fh \quad (1)$$

where Fa , Fg , and Fh are fraction absorbed, intestinal availability, and hepatic availability, respectively.

Although it is thought that Fa and Fh predominantly contributes to F , Fg shows a significant contribution in the case of some CYP3A4 substrates such as cyclosporine, midazolam, sirolimus, and nifedipine¹⁻⁴⁾ and some CYP3A4 substrates exhibit intestinal first-pass metabolism.

A drug which is metabolized by a pathway exhibiting an intestinal first-pass effect may show great individual differences in pharmacokinetics. There is also the possibility of severe drug-drug interactions when an inhibitor or inducer is co-administered because not only hepatic CYP3A4 but also intestinal CYP3A4 is inhibited or induced by the inhibitor or inducer. Therefore, characterization of the intestinal first-pass metabolism of a drug is important.

Not only CYP3A4 but also P-glycoprotein (P-gp) is present in the small intestine acting as an efflux pump for drugs and presenting a barrier to drug absorption.^{5,6)} It is not obvious which CYP3A4 or P-gp plays a role in low bioavailability because of the overlapping substrate specificity of CYP3A4 and P-gp.⁷⁾ Benet *et al.* have proposed that the

synergistic effects of CYP3A4-mediated metabolism and P-gp-mediated efflux in the intestine may result in unexpectedly high first-pass metabolism.^{8,9)} The role of P-gp and its synergistic effects are important but very complex. Therefore, in this review, the focus is on the intestinal first-pass metabolism of CYP3A4 substrates.

Intestinal CYP3A4

Enterocytes have the ability to metabolize drugs by numerous pathways involving phase I and II reactions.^{9,10)} Zhang *et al.* demonstrated the expression of CYP1A1, 1B1, 2C, 2D6, 2E1, 3A4, and 3A5 mRNAs in enterocytes in human. However, from Western blot analysis, they did not detect CYP1B1, 2D6, 2E1, and 3A5 proteins, but found only CYP3A4 and 2C in human enterocyte microsomes.¹¹⁾ Similarly, Paine *et al.* applied Western blot analysis to microsomes prepared from mucosal scrapings from the duodenal/jejunal portion of 31 human donor small intestines.¹²⁾ They demonstrated that CYP3A and CYP2C9 represent the majority of the intestinal P450 pie, accounting for 80% and 15% of the total immunoquantified P450s, respectively. They also found that the expression of CYP3A4 varies along the length of the small intestine. Median concentrations of microsomal protein CYP3A4 in human duodenum, distal jejunum, and distal ileum were 32, 23, and 17 pmol/mg, respectively.¹³⁾ The total amount of intestinal CYP3A reported by Paine *et al.*¹²⁾ and by von Richter *et al.*¹⁴⁾ was 70.5 and 65.7 nmol, respectively. Therefore, the CYP3A4 content in the intestine is estimated to be less than 1% of that in the

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Abbreviations: AUC, area under the curve; CYP, cytochrome P450; Fg , intestinal availability; Fh , hepatic availability; Fa , fraction absorbed; P-glycoprotein, P-gp.

liver.

The cDNA sequence of CYP3A4 obtained from the intestine was the same as that from the liver,¹⁵⁾ and so the CYP3A4 proteins in the liver and intestine should be the same protein. In addition, reported K_m values of CYP3A4 for some substrates in the liver and intestine were similar.^{2,16,17)} Therefore, the ratio of CYP3A4 in the liver to that in the intestine is considered to be the ratio of the intrinsic clearance between liver and intestine for CYP3A4 substrates. The mean value for the ratios reported previously was 8.1.¹⁷⁾

The estimation of F_g in human

The F_g of CYP3A4 substrates in human could be estimated using the following equations:

$$CL_h = CL_{tot} - CL_r \quad (2)$$

$$F_h = 1 - CL_h / Q_h \quad (3)$$

$$F_a \cdot F_g = F / F_h \quad (4)$$

where CL_h , CL_{tot} , CL_r , and Q_h are hepatic clearance, total body clearance, renal clearance, and hepatic blood flow rate, respectively. F_a and F_g were evaluated as the product $F_a F_g$ because F_a and F_g cannot be separated.

Lin *et al.* have highlighted a number of problems associated with the estimation of intestinal first-pass metabolism.⁹⁾

One problem is that F_g is estimated indirectly under the assumption that the drug is systemically metabolized only in the liver (i.e. F_a is 1).⁹⁾ In addition, it has been pointed out that F_g , obtained by dividing F by F_h , is influenced by the rate of hepatic blood flow.⁹⁾ Hepatic clearance (CL_h) and hepatic blood flow rate (Q_h) are needed to calculate F_h . When $CL_h = 600$ mL/min, $F = 0.5$, and $Q_h = 1200$ mL/min, the value of F_g is 1. If $Q_h = 1600$ mL/min, $F_g = 0.8$ and so F_g depends on Q_h .

Kato *et al.*¹⁸⁾ evaluated the effect of Q_h on $F_a F_g$ values for ten compounds metabolized by CYP3A in humans using three Q_h values (17.1, 21.4, and 25.5 mL/min/kg). **Figure 1** shows the relationship between F_h and $F_a F_g$ using the three Q_h values. The F_h of four CYP3A4 substrates (indinavir, nicardipine, nifedipine, and verapamil) for which the hepatic clearance was more than 8 mL/min/kg was influenced by the Q_h (**Fig. 1**). Therefore, the $F_a F_g$ of those substrates was also influenced by the Q_h (**Fig. 1**). The $F_a F_g$ values for nicardipine, felodipine, midazolam, cyclosporine, and tacrolimus were less than 0.5 for all rates of hepatic blood flow. If the F_a of these substrates of CYP3A4 can be obtained, it is possible to estimate their intestinal first-pass metabolism. In animal and human studies using labeled compounds, nicardipine, felodipine, and nifedipine were completely absorbed,¹⁹⁻²¹⁾ and their low values of $F_a F_g$ were possibly due to intestinal first pass metabolism. Information

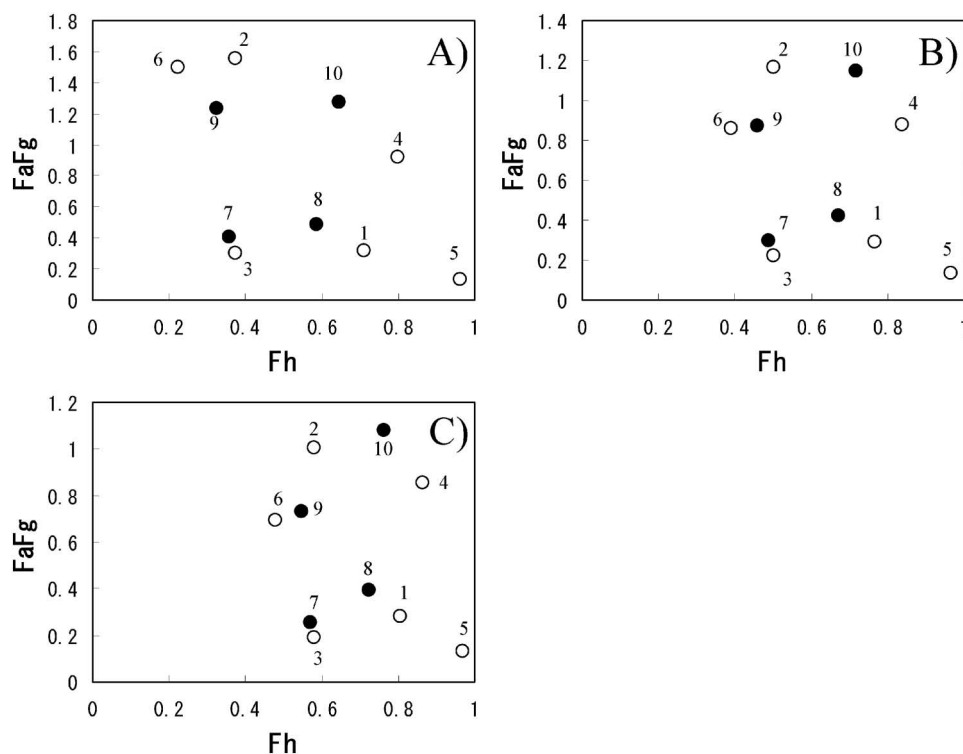


Fig. 1. Relationship between F_h and $F_a F_g$ of the substrates of CYP3A4 (Ref. 18)

F_h and $F_a F_g$ were calculated using hepatic blood flow rates in mL/min/kg of 17.1 (A), 21.4, (B) and 25.5 (C). ○: P-gp substrates. ●: non P-gp substrates. 1, cyclosporine; 2, indinavir; 3, nicardipine; 4, quinidine; 5, tacrolimus; 6, verapamil; 7, felodipine; 8, midazolam; 9, nifedipine; 10, propafenone.

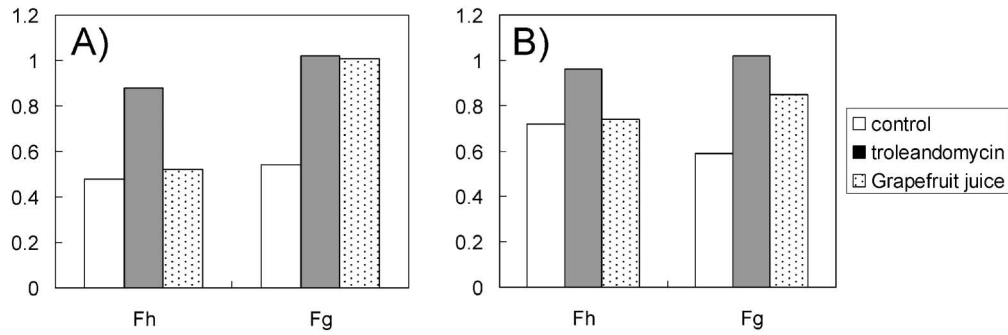


Fig. 2. Effect of CYP3A4 inhibitors (troleandomycin and grapefruit juice) on Fh and FaFg of midazolam (A) and alfentanil (B) illustrated based on the data of ref. 22.

on *Fa* from absorption studies using a labeled compound is needed for the estimation of *Fg*.

Enzyme inhibition

Many interactions mediated by the inhibition of CYP3A4 have been reported. It is not so obvious which inhibitions, hepatic and/or intestinal CYP3A4, contribute to the drug-drug interaction is not so obvious. However, estimations of the *Fh* and *FaFg* of a substrate with and without an inhibitor can be clarified using the estimation described above. **Figure 2** shows the *Fh* and *FaFg* of midazolam and alfentanil with and without co-administration of troleandomycin and grapefruit juice.²²⁾ Co-administration of troleandomycin with midazolam and alfentanil caused an increase in *Fh* and *FaFg*, suggesting that troleandomycin inhibited intestinal and hepatic CYP3A4. On the other hand, grapefruit juice did not change the *Fh* of midazolam or alfentanil and increased the *FaFg* of midazolam and alfentanil. These results suggest that grapefruit juice inhibited the intestinal CYP3A4 but not the hepatic CYP3A4. In addition, grapefruit juice had no effect on the plasma profiles of cyclosporine,²³⁾ felodipine,²⁴⁾ or saquinavir²⁵⁾ after intravenous administration, while after oral administration, grapefruit juice increased the AUC of these drugs. The finding also suggests that grapefruit juice is a selective inhibitor for intestinal CYP3A4. **Table 1** summarizes the interactions of orally administered drugs with grapefruit juice. In accordance with the discussion described above, the least change in the AUC by the co-administration of grapefruit juice was found in quinidine which exhibits high bioavailability and low intestinal metabolism.²⁹⁾ Therefore, increase in the AUC of CYP3A4 substrates by the co-administration of grapefruit juice might be an index for intestinal first-pass metabolism, although it cannot be denied that the increase may be due to the inhibition of an efflux transporter such as P-glycoprotein.

Ketoconazole and itraconazole also caused an increase in the AUC of CYP3A4 substrates (**Table 2**). For alprazolam and quinidine with high *Fg* values, co-administration of 200 mg of itraconazole caused respective increases in their AUCs of 2.7- and 2.4-fold. Co-administration of midazolam

Table 1. Interaction of grapefruit juice and CYP3A4 substrates

Grapefruit juice intake	Substrate	AUC (fold increase)	<i>C_{max}</i> (fold increase)	Ref. no.
400 mL	Felodipine	3.34	2.91	26
400 mL	Nifedipine	1.58	1.16	27
250 mL	Sildenafil	1.23	0.96	28
248.8 g	Quinidine	1.08	0.93	29
400 mL	Atorvastatin	2.46	1.06	30
400 mL	Lovastatin	15.26	11.77	31
400 mL	Simvastatin	16.14	9.42	32
200 mL	Midazolam	1.52	1.56	33
250 mL	Triazolam	1.48	1.25	34
400 mL	Buspirone	9.21	4.29	35

Table 2. Drug-drug interaction of CYP3A4 substrates and azoles

Inhibitor	Dose (mg)	Substrate	AUC (fold increase)	<i>C_{max}</i> (fold increase)	Ref. no.
Itraconazole	200	Alprazolam	2.7	1.3	36
	200	Atorvastatin	3.3	1.2	37
	200	Atorvastatin	2.5	1.4	38
	100	Buspirone	19.2	13.4	39
	200	Felodipine	6.3	7.8	40
	200	Lovastatin	22.1	13.1	41
	100	Lovastatin	8.6	9.7	42
	200	Midazolam	10.8	3.4	43
	100	Midazolam	5.8	2.6	44
	200	Midazolam	8.0	3.1	45
	200	Quinidine	2.4	1.6	46
	200	Simvastatin	18.6	16.7	47
	200	Triazolam	27.1	2.8	48
200	Triazolam	3.1	1.4	49	
200	Zolpidem	1.3	1.1	50	
Ketoconazole	200	Alprazolam	4.0	1.1	51
	200	Alprazolam	1.8	1.1	52
	200	Cyclosporine	5.3	2.0	53
	400	Midazolam	15.9	4.1	43
	200	Midazolam	15.1	4.2	54
	200	Midazolam	8.1	3.1	55
	400	Tacrolims	2.4	2.0	56
	400	Triazolam	22.0	3.0	48
200	Zolpidem	1.7	1.3	57	

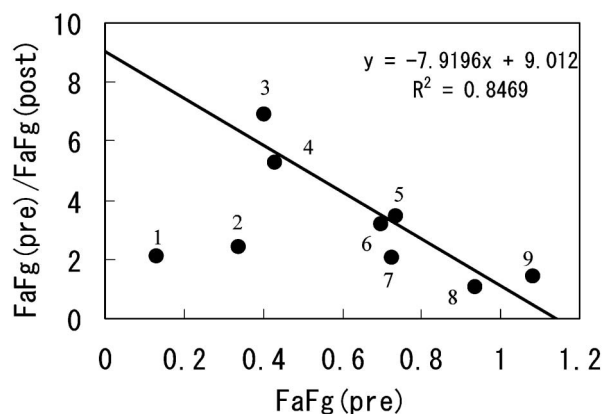


Fig. 3. Relationship between $FaFg(\text{pre})$ and $FaFg(\text{pre})/FaFg(\text{post})$ of substrates of CYP3A4 (Ref. 18)

$FaFg(\text{pre})$ and $FaFg(\text{post})$ are $FaFg$ before and after rifampicin treatment. $FaFg$ were calculated using a hepatic blood flow rate of 25.5 mL/min/kg. 1, tacrolimus; 2, cyclosporine; 3, midazolam; 4, triazolam; 5, nifedipine; 6, verapamil; 7, zolpidem; 8, quinidine; 9, propafenone.

and itraconazole caused an 8- to 10-fold increase in the AUC of midazolam. Co-administration of a strong inhibitor such as ketoconazole and itraconazole likely caused the remarkable increase in AUC of CYP3A4 substrates such as midazolam, triazolam, and simvastatin with low Fg values.

Enzyme induction

Multiple oral administrations of rifampicin caused induction of CYP3A4 in the liver and intestine. Although rifampicin induces CYP3A4, it also induces P-gp.⁵⁸⁾ P-gp and CYP3A4 content in biopsy samples after treatment with rifampicin (600 mg/day) measured by Western blotting showed increases of 3.5- and 4.4-fold, respectively, as compared with the values before treatment.⁵⁹⁾ Treatment with rifampicin caused 27 and 17% reduction in the AUCs of zolpidem⁶⁰⁾ and quinidine,⁶¹⁾ respectively, both of which exhibited high bioavailability. On the contrary, the AUCs of alfentanil, midazolam,²²⁾ and triazolam,⁶²⁾ which undergo first-pass intestinal metabolism, were reduced to 1/20, 1/20, and 1/30 of the control by the treatment with rifampicin, respectively. The relationship between $FaFg$ before 600 mg/day treatment with rifampicin and the ratio of $FaFg$ before treatment to that after treatment was previously reported (Fig. 3).¹⁸⁾ A negative correlation between $FaFg$ and the ratio of $FaFg$ before treatment with rifampicin to that after treatment was observed except for cyclosporine and tacrolimus. The smaller the value of $FaFg$, the greater the effect of rifampicin. Co-administration of an inducer such as rifampicin is likely the cause of the remarkable reduction in the AUCs of CYP3A4 substrates with low Fg values such as midazolam and triazolam.

Saturation of intestinal metabolism

The concentration in the intestine might be higher than

unbound concentration in circulation, which would cause the saturation to be greater for intestinal metabolism than for hepatic metabolism. The pharmacokinetics of midazolam were investigated following oral administration of 7.5, 15 and 30 mg doses of midazolam in solution to healthy subjects.⁶³⁾ The C_{max} and AUC increased more than proportionally after the 30 mg dose. No significant difference among doses was observed in the half-life of midazolam.

Sildenafil also exhibited nonlinear kinetics.⁶⁴⁾ After oral administration of doses ranging from 25 to 200 mg, systemic exposure of sildenafil increased slightly more than dose proportionally. Nonlinearity was more pronounced for doses greater than 200 mg. An 8-fold increase in dose from 100 to 800 mg resulted in 15- and 14-fold increases in AUC and C_{max} , respectively. In this case, the unbound maximum concentration at the inlet to the liver ($C_{inlet, max}$) of midazolam and sildenafil could be calculated to evaluate the saturation of CYP3A4 in the liver using the pharmacokinetic parameters from the literature^{2,63,64)} and the following equation:

$$C_{inlet, max} = C_{max, u} + fu \left(\frac{ka \cdot Fa \cdot Dose}{Qh} \right) \quad (5)$$

where $C_{max, u}$, fu , and ka , are maximum blood concentration, blood unbound fraction, absorption rate constant, respectively. When ka , Fa , and Qh values were assumed to be 0.1 min^{-1} , 1, and 1610 mL/min, respectively, the $C_{inlet, max}$ values for midazolam (30 mg) and sildenafil (200 mg) are calculated to be 0.13 and 1.1 mcM, respectively. Since the Michaelis constants used for midazolam and sildenafil are 1.9⁶⁵⁾ and 14.4 mcM,⁶⁶⁾ the $C_{inlet, max}$ values are lower than the Michaelis constants for these drugs. Therefore, the nonlinear kinetics of these drugs might be due to the saturation of intestinal metabolism.

Prediction of intestinal metabolism

A drug with low bioavailability due to intestinal first-pass metabolism can be affected by the enzyme inhibition, induction, and saturation, which may result in widely variable pharmacokinetics. Therefore, it is preferable to select a candidate that exhibits no intestinal first-pass metabolism during the drug discovery and development process, whereas the prediction of intestinal first-pass metabolism is of important.

Well-stirred, parallel tube, and dispersion models have been used for the prediction of hepatic availability. However, no prediction model for intestinal availability has been established even though several models have been reported as follows.

In some reports, the well-stirred model was applied to predict the intestinal extraction ratio (Eg) in rats,⁶⁷⁻⁶⁹⁾ and is estimated by the following equation:

$$Eg = \frac{fu \cdot CL_{int}}{Q + fu \cdot CL_{int}} \quad (6)$$

where Q , CL_{int} and fu are blood flow rate, intestinal intrinsic clearance, and unbound fraction in enterocytes. This model does not include a parameter of absorption such as membrane permeation and the total intestinal or mucosal blood flow rate has been used as the blood flow rate. Some reports succeeded and other failed.

De Vries, M *et al.*⁷⁰⁾ predicted the Eg of 1-naphthol from a perfusion study using the following equation:

$$Eg = \frac{CL_{int}}{CL_{int} + CL_{abs}} \quad (7)$$

where CL_{abs} is the absorption clearance. The estimated Eg was comparable to that of *in vivo* data.

Shen *et al.*⁷¹⁾ estimated the Eg of 11 CYP3A4 substrates and the 2D6 substrate using the following equation:

$$Eg = \frac{CL_{int}}{fv \cdot Q_{sma} + CL_{int}} \quad (8)$$

where $fv \cdot Q_{sma}$ is the mucosal blood flow rate. This equation does not include the unbound fraction and the accuracy of the predicted Eg for the 12 compounds was poor.

Yang *et al.*⁷²⁾ used the “ Q_{gut} ” model which retains the form of the well-stirred model. Q_{gut} is a hybrid of both permeability through the enterocyte membrane (CL_{perm}) and villous blood flow (Q_{villi}):

$$Fg = \frac{Q_{gut}}{Q_{gut} + fu_G \cdot CL_{int}} \quad (9)$$

$$Q_{gut} = \frac{Q_{villi} \cdot CL_{perm}}{Q_{villi} + CL_{perm}}$$

where fu_G is the unbound fraction in the enterocyte. They compared the predictive accuracy of the well-stirred model with that of the Q_{gut} model. In the case of the well-stirred model, the predicted Fg values were overestimated. With the Q_{gut} model, when fu_G is unity, the predicted Fg values were similar to actual Fg values.

Fagerholm⁷³⁾ proposed a simple method whereby the Eg is predicted from the ratio of the CL_{int} of a reference compound to that of the test compound:

$$Eg = E_{g,ref} \cdot (CL_{int}/CL_{int,ref}) \cdot fa \quad (10)$$

where

$$Eg = E_{g,ref} \cdot (CL_{int}/CL_{int,ref}) > 1 \text{ is set to unity}$$

where $E_{g,ref}$ and $CL_{int,ref}$ are the first pass *in vivo* Eg and *in vitro* CL_{int} for a reference compound with high permeability, respectively, and CL_{int} and fa are the *in vitro* CL_{int} and predicted fraction of absorbed drug for a test compound, respectively. The predicted Eg values of 4 compounds using the CL_{int} of verapamil as the reference compound were similar to the observed values. Equation 9 does not include the

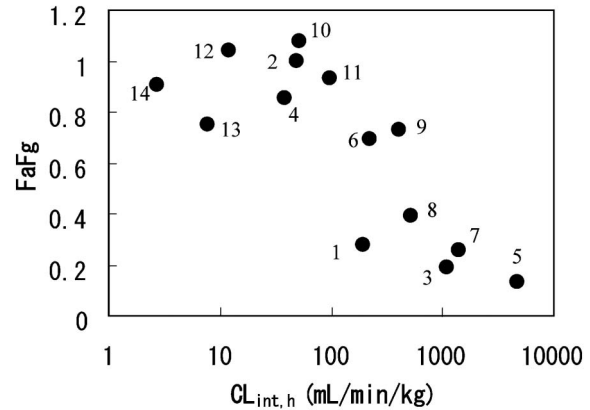


Fig. 4. Relationship between CL_{int} and the $FaFg$ of substrates of CYP3A4 (Ref. 18)

$FaFg$ was calculated using a hepatic blood flow rate of 25.5 mL/min/kg. 1, cyclosporine; 2, indinavir; 3, nifedipine; 4, quinidine; 5, tacrolimus; 6, verapamil; 7, felodipine; 8, midazolam; 9, nifedipine; 10, propafenone; 11, amlodipine; 12, clonazepam; 13, carbamazepine; 14, alprazolam.

unbound fraction.

Finally, Kato *et al.*¹⁸⁾ reported a semi-quantitative prediction method from the relationship between *in vivo* hepatic intrinsic clearance and $FaFg$ (Fig. 4). $FaFg$ was markedly reduced when the hepatic intrinsic clearance was more than 100 mL/min/kg. This phenomenon was not observed in any of the substrates of CYP isoforms other than CYP3A4. The use of hepatic intrinsic clearance (100 mL/min/kg) was proposed as the index of intestinal first-pass metabolism.

In the prediction methods described here, the predictability was high when unbound fraction was 1. However, the parameters such as intestinal intrinsic clearance, blood flow rate, permeability, and unbound fraction are necessary for the accurate prediction of the intestinal first-pass metabolism of a drug. In addition, it is necessary to develop a prediction model containing the efflux of P-gp because of the overlapping substrate specificity of CYP3A4 and P-gp.

Conclusion

CYP3A4 is present in the small intestine and functions as a barrier against xenobiotics. Some CYP3A4 substrates exhibit low bioavailability due to intestinal first-pass metabolism. The AUCs of such CYP3A4 substrates are remarkably changed by the inhibition, induction, and saturation of CYP3A4. Although some prediction methods for intestinal first-pass metabolism have been reported, a standard method has not been established. More studies are needed to establish a standard method for predicting intestinal first-pass metabolism.

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