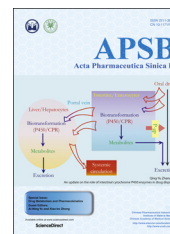




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REVIEW

An update on the role of intestinal cytochrome P450 enzymes in drug disposition



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Abstract Oral administration is the most commonly used route for drug treatment. Intestinal cytochrome P450 (CYP)-mediated metabolism can eliminate a large proportion of some orally administered drugs before they reach systemic circulation, while leaving the passage of other drugs unimpeded. A better understanding of the ability of intestinal P450 enzymes to metabolize various clinical drugs in both humans and preclinical animal species, including the identification of the CYP enzymes expressed, their regulation, and the relative importance of intestinal metabolism compared to hepatic metabolism, is important for improving bioavailability of current drugs and new drugs in development. Here, we briefly review the expression of drug-metabolizing P450 enzymes in the small intestine of humans and several preclinical animal species, and provide an update of the various factors or events that regulate intestinal P450 expression, including a cross talk between the liver and the intestine. We further compare various clinical and preclinical approaches for assessing the impact of intestinal drug metabolism on bioavailability, and discuss the utility of the intestinal epithelium-specific NADPH-cytochrome P450 reductase-null (IECN) mouse as a useful model for studying *in vivo* roles of intestinal P450 in the disposition of orally administered drugs.

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Abbreviations: AUC, area under concentration-time curve; CPR, NADPH-cytochrome P450 reductase; DDI, drug–drug interaction; GFJ, grapefruit juice; IECN, intestinal epithelium-specific *Cpr*-null; LCN, liver-specific *Cpr*-null; P450 (or CYP), cytochrome P450; P-gp, P-glycoprotein; WT, wide-type

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1. Introduction

Oral administration is the most commonly used route for drug treatment because of the advantages of a lower cost and easier compliance by patients, compared to other routes, particularly for chronic treatment. However, a low oral bioavailability would make oral dosing less desirable or practical for many drugs. Evaluation of oral bioavailability of drug candidates, which is usually performed during the drug discovery and preclinical drug development stages, is crucial for strategic decision-making. Cumulative data have demonstrated that intestinal cytochrome P450 (CYP)-mediated metabolism can eliminate a large proportion of some orally administered drugs before they reach systemic circulation, while leaving the passage of other drugs unimpeded. Drugs that are subject to high intestinal metabolism not only suffer from low bioavailability, but they are also more likely to be susceptible to drug–drug interactions (DDI) with other P450 substrate or inducer drugs and show large inter-individual variations in pharmacokinetic profiles. Therefore, a better understanding of the ability of intestinal P450 enzymes to metabolize various clinical drugs in both humans and preclinical animal species, including the identification of the CYP enzymes expressed, their regulation, and, at a systems level, the relative importance of the liver and the intestine in the first-pass metabolism and disposition of oral drugs, is important for improving bioavailability of current drugs and new drugs in development.

The topics of intestinal P450 expression, regulation, and function in drug metabolism have been reviewed previously^{1–4}. This brief update will review more recent advances in the field while summarizing earlier findings, with a special focus on approaches available to assess the specific contributions by intestinal P450-mediated drug metabolism to first-pass drug disposition and the impact on bioavailability.

2. Expression of drug-metabolizing CYPs in the intestine

The ability of the intestine to metabolize numerous drugs and other xenobiotics is defined to a large extent by the type and abundance of the individual CYP enzymes expressed in the tissue. Therefore, large efforts have been made to detect and quantify the various CYP isoforms in the intestine of both humans and experimental animals.

2.1. CYP expression in human intestine

The human small intestine expresses multiple CYP genes, as has been reviewed previously^{1,2}. For example, in human small intestinal epithelial cells (enterocytes) prepared using an elution method with an EDTA-containing buffer, which mostly consists of villous enterocytes, with little crypt cell contamination, *CYP1A1*, *CYP1B1*, *CYP2C*, *CYP2D6*, *CYP2E1*, *CYP3A4*, and *CYP3A5* mRNAs were detected, although a number of other CYP transcripts, including *CYP1A2*, *CYP2A6*, *CYP2A7*, *CYP2B6*, *CYP2F1*, *CYP3A7*, and *CYP4B1*, were not detected⁵. The expression of CYP1A1, 2C, and 3A4 proteins was also confirmed *via* immunoblot analysis. An immunoblot study of microsomes prepared from mucosal scrapings from the duodenal/jejunal portion of human donor small intestines indicated that CYP3A (CYP3A4 and 3A5) and CYP2C9 represent the major constituents of the intestinal “P450 pie”, accounting for 80% and 14%, respectively, of total immuno-quantified P450s⁶. CYP3A4, which was the main CYP3A

protein detected, was found in all individuals analyzed; whereas CYP3A5 was only detected in some individuals, where they represented 3%–50% of total CYP3A content. The remaining detected CYP enzymes had the following rank order: CYP2C19 > 2J2 > 2D6.

There are large interindividual variations in the expression levels of individual P450s. For example, the levels of CYP2C9 and 2C19 proteins in small intestine were determined to be, on average, 14% and 2%, respectively, of total P450 in the intestine; but interindividual differences were 9-fold for CYP2C9 and 6.5-fold for CYP2C19⁶. An earlier study using metabolic activities to monitor the expression of different CYP2C isoforms in the human small intestine (diclofenac 4'-hydroxylase for CYP2C9 and mephenytoin 4'-hydroxylase for CYP2C19), showed 17–18-fold differences for these CYPs among the intestines investigated⁷.

Of the less abundant CYP enzymes in the intestine, CYP2J2 has been studied intensively^{8,9}. Although CYP2J2 is recognized mainly for its ability to catalyze arachidonic acid metabolism, it also metabolizes many structurally diverse drugs, such as terfenadine, astemizole, amiodarone and tamoxifen¹⁰.

Another P450 with a somewhat preferential expression in the intestine is CYP2S1¹¹. CYP2S1 has been shown to be capable of activating the anti-cancer prodrug 1,4-bis[[2-(dimethylamino-*N*-oxide)ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione (AQ4N) through reductive metabolism^{12,13}, and to reduce the *N*-hydroxylamine drug 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole¹⁴.

Several studies have examined developmental expression of CYPs in the human intestine^{15–18}. The orphan P450 CYP2W1 is expressed in fetal intestine, but its expression is suppressed soon after birth¹⁵. CYP2C and CYP2J2 are expressed in human fetal intestine at an early stage, and the fetal intestinal level of CYP2J2 is apparently higher than the level in adult intestine¹⁸. CYP3A4 is expressed in both prenatal and postnatal intestine; its expression level in neonatal duodenal tissue increased with age^{16,17}. The ability of human fetal intestine to metabolize drugs has not been examined.

2.2. CYP expression in mouse small intestine

Most studies on CYP expression in experimental animals were conducted with rodents, particularly mice, as have been reviewed previously¹. Mice are widely used in preclinical studies and in the development of transgenic, knockout, and humanized mouse models. Mice have a greater number of *Cyp* genes (102 genes) than do humans (57 genes), which contributes to the species differences between mice and humans in drug metabolism. Many, but not all, of the CYPs that are expressed in liver are also expressed in the small intestine. Early studies on the expression of mouse intestinal CYPs relied on RNA-PCR, immunoblotting, and activity measurements^{19–25}. Many isoforms, including CYP1A1, 1B1, 2B9, 2B10, 2B19, 2C29, 2C38, 2C40, 2E1, 2J6, 3A11, 3A13, 3A16, 3A25, and 3A44, were identified, whereas several others, including CYP1A2, 2A, 2C37, 2C39, and 2F2, were not detected. A screening assay for all CYPs of the *Cyp1–4* families in adult male and female C57BL/6 mice showed that the mRNAs for ~10% of these genes were expressed at the highest levels in the small intestine, compared to 13 other tissues, including the liver²⁶. A recent study also profiled mouse intestinal CYP protein expression using a mass spectrometry-based proteomics approach, which detected a total of 27 proteins belonging to P450 subfamilies 1A, 2A, 2B, 2C, 2D, 2E, 2F, 2J 2U, 3A, 4A, 4B, 4F, and

4V in various tissues, of which CYP2C29, 2C37, 2J5, 3A13, 3A25, 4A12, 4A10 and 4B1 were detected in the intestine²⁷.

2.3. CYP expression in the small intestine of non-human primates

In the cynomolgus monkey, which is evolutionarily closer than rodents are to humans, mRNA expression levels of multiple CYPs in the CYP1–3 families showed regional differences along the length of the small intestine²⁸; regional differences in microsomal activity toward model CYP substrates were also observed, with CYP3A activities (midazolam 1'-hydroxylation and testosterone 6 β -hydroxylation) showing a decrease from jejunum to ileum²⁹. Species differences between monkeys and humans in intestinal drug metabolism have been noted, with cynomolgus monkeys having greater intestinal activity toward human CYP3A, CYP2C, and CYP2J substrates, and with the activity toward human CYP2C/CYP2J substrates apparently attributed to monkey CYP2C and CYP4F^{30,31}. Further studies showed additional differences in not only intestinal microsomal activities, but also inhibitor selectivity between monkeys and humans³². The contents of specific P450 proteins of CYP1–4 families in monkey small intestine were estimated using selective anti-CYP antibodies; the results from pooled microsomes suggested that CYP3A and CYP4F were the most abundant, followed in decreasing order by CYP2J, CYP2C, CYP1A and CYP2D. CYP protein levels varied by 2–10 folds among microsomal samples from individual monkeys^{33,34}. Several studies also characterized intestinal CYP expression in the common marmosets, another species of non-human primate^{35–37}.

2.4. CYP expression in the small intestine of other animal species

Intestinal CYP expression has also been studied in other animal species commonly used in preclinical drug development, such as rats^{1,38–40} and dogs^{38,41,42}. There is an overall conservation in the major CYP subfamilies that are expressed in the intestine, such as CYP3A and CYP2C. However, among species, the quantitative aspects regarding relative levels of a given isoform among all intestinal P450s, or compared to human intestinal expression levels, can be different; the numbers of CYP (*Cyp*) genes within each CYP subfamily are often different; and it is not always possible to identify orthologs, particularly for members of the CYP2–4 families, as the substrate specificity of some seemingly orthologous isoforms from different species can be different. For example, it has been posited that the rat is not an ideal animal model for predicting intestinal loss of drugs during presystemic metabolism⁴⁰, for several reasons: a higher bioavailability was achieved in humans than in rats for ~75% orally administered compounds; for CYP3A, the concentration in human intestinal microsomes was much higher than in rat intestinal microsomes^{6,43}; and the intestinal CYP3A activities towards representative CYP3A substrates were different by 2–5 folds between humans and rats³¹. Details of such species difference are important for drug development efforts, as the selection of animal species for preclinical study is often dictated by efficacy and/or safety profiles of drug candidates; therefore, it is important to understand the intestinal drug metabolism properties of the chosen species.

3. Regulation of intestinal CYP expression and function

Given the well-recognized capability of intestinal P450 enzymes to metabolize orally administered drugs and other xenobiotics, it is conceivable that factors or events that alter the expression or activity of intestinal P450 enzymes could significantly impact the first-pass drug clearance in this portal-of-entry organ. A wide variety of drugs, other xenobiotics, and food components, including dietary phytochemicals, may influence intestinal drug disposition via induction or inhibition of intestinal CYP expression and/or activity. For examples, in mouse intestine, CYP1A1 was greatly induced by β -naphthoflavone; all five CYP3A isoforms were induced by dexamethasone; CYP2B9, and CYP2B10 were induced, whereas CYP2B19 mRNA level was much reduced, by phenobarbital treatment; CYP2C29 and CYP2C40 were also induced by phenobarbital while CYP2C38 showed no induction²⁴; and CYP2J6 was induced by pyrazole²³. Recent developments in this area are highlighted below, with a focus on dietary and physiological regulations.

3.1. Regulation by drugs, herbs, pathogens and disease conditions

These studies are mainly conducted in rodent models and/or human intestinal cells. Mouse intestinal CYP1A1 expression, both basal and benzo[*a*]pyrene-induced, was found to be dependent on the presence of a functional Toll-like receptor 2, which is important in pathogen recognition and innate immunity in the gut⁴⁴. Mouse intestinal CYP1A1 and CYP2B10 were both induced by repeated oral administration of the antiparasitic drug ivermectin⁴⁵. Mouse intestinal CYP3A expression was suppressed by insulin treatment⁴⁶, although the effects of experimental models of type I and type II diabetes on intestinal CYP3A expression or activity seemed contradicting in two different studies^{46,47}. Furthermore, treatment of monosodium glutamate-induced obese mice with green tea extract decreased insulin level, and increased the expression of CYP3A in both liver and small intestine⁴⁸. Human CYP3A4 was induced by 3,3'-diindolylmethane, a herbal nutritional supplement, and piperine, a black pepper constituent, in human intestinal cells *via* PXR^{49,50}. On the other hand, induction of CYP3A4 by 1 α ,25-dihydroxyvitamin D3 in Caco-2 cells was inhibited by andrographolide, another herbal ingredient⁵¹. In rats, intestinal CYP3A1 expression was increased by the plasticizer acetyl tributyl citrate; the latter also increased CYP3A4 expression in human intestinal cells⁵². Intestinal expression of several rat CYPs, including CYP1A1, CYP2E1, and CYP3A9, was suppressed by treatment of rats with probiotic *Lactobacillus casei*⁵³, and intestinal expression of CYP3A was decreased in rats treated with the probiotic *Escherichia coli* Nissle 1917⁵⁴. The effects of intestinal inflammation on CYP expression have also been examined. In a mouse model of dextran sodium sulfate-induced colitis, CYP3A as well as P-gp expression was down-regulated in the upper part of small intestine⁵⁵. In a rat model of indomethacin-induced intestinal ulcers, a small decrease in CYP2D2 expression was found in the upper part of the small intestine⁵⁶.

3.2. Dietary regulation of CYP expression or activity in mouse intestine

A well-known example of dietary inhibitors of CYP activity is grapefruit juice (GFJ). GFJ, when administered together with

either nifedipine or felodipine, increases the plasma concentration of the drug⁵⁷. The metabolism of numerous drugs, including coumarin, cyclosporine, ethinylestradiol, midazolam, terfenadine, and verapamil^{58,59}, saquinavir^{60,61}, and erythromycin⁶², was also shown to be decreased by GFJ. The GFJ-mediated decrease in substrate metabolism occurs through a mechanism-based inactivation of enterocyte CYP3A4, possibly by furanocoumarin constituents of GFJ⁶³. Notably, orally ingested GFJ did not seem to affect hepatic CYP3A4 expression or activity, while decreasing small-intestinal CYP3A4 protein levels by >60%⁶⁴. In contrast, the consumption of cranberry extract, which caused moderate increases in hepatic CYP3A and CYP1A1 activities, did not influence intestinal CYP1A1, CYP1A2, CYP2B and CYP3A expression in rats⁶⁵. GFJ has been employed as a tool to modulate intestinal drug metabolism in numerous studies. For example, in a recent study of intestinal P450 contribution to intestinal toxicity induced by oral diclofenac (DCF), a nonsteroidal anti-inflammatory drug, GFJ extract inhibited the *in vitro* bioactivation of DCF by mouse and human intestinal microsomes, and decreased the extent of DCF-induced intestinal injury in mice, a finding suggested potential utility of GFJ in the protection against DCF-induced SI toxicity in patients⁶⁶.

Potential dietary regulation of intestinal drug metabolism is also illustrated by the effects of a synthetic diet on intestinal P450 expression and function⁶⁷. When mice maintained on a regular laboratory chow diet were fed a synthetic (albeit nutritionally balanced) diet devoid of phytochemicals, they exhibited diminished intestinal expression of CYP1A, 2B, 2C, and 3A and hepatic expression of CYP2B, 2C, and 3A. These reductions in P450 expression were accompanied by decreases in microsomal metabolism of midazolam, a CYP3A substrate, and first-pass clearance of midazolam *in vivo* in wild-type (WT) mice.

3.3. Regulation of intestinal CYP expression by hepatic P450 activity

Xenobiotics that are absorbed by intestinal enterocytes and escape the metabolic disposition by intestinal P450 will likely reach liver *via* the portal vein (Fig. 1), and may then be metabolized by hepatic P450 enzymes. Thus, P450 expression in the liver and intestine may be coordinately regulated, in a way that helps to maintain the overall metabolic capacity of the digestive organs for first-pass clearance of ingested compounds. This hypothesis was derived from a study of CYP expression in the liver-specific *Cpr*-null (LCN) mouse model,

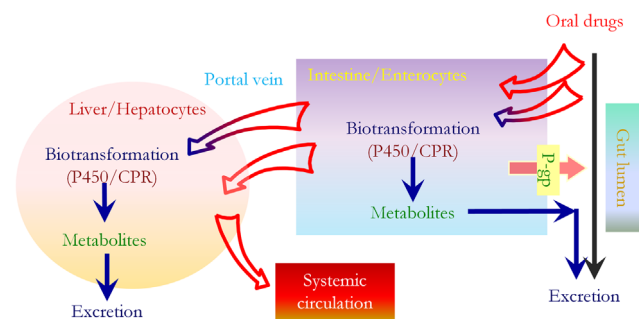


Figure 1 Sequential action of intestinal and hepatic P450 enzymes on orally ingested drugs. Absorbed drugs that escape metabolism by intestinal P450 and disposition by efflux transporters (*e.g.*, P-glycoprotein; P-gp) may be metabolized by hepatic P450 on their way to systemic circulation.

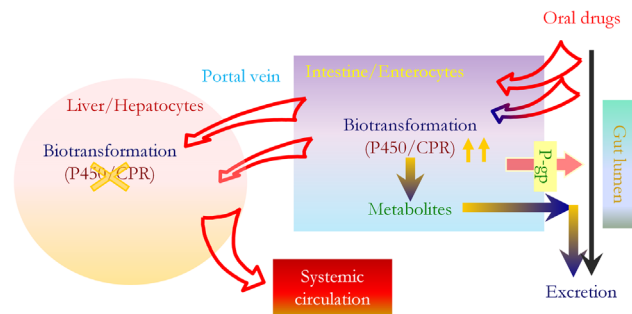


Figure 2 Regulation of intestinal P450 expression by hepatic P450 activity. The loss of hepatic P450 activity in the LCN mouse leads to increased amounts of the un-metabolized drugs entering systemic circulation, as well as upregulation of intestinal P450 expression and increased contribution of intestinal P450 enzymes to first-pass metabolism of orally ingested drugs.

in which the activities of all microsomal P450s are suppressed in the hepatocytes due to deletion of the *Cpr* gene⁶⁸. The loss of hepatocyte *Cpr* caused compensatory increases (2–3 folds) in intestinal expression of CYP2B, 2C and 3A proteins in the LCN mice, compared with WT mice, accompanied by significant augmentations of intestinal microsomal lovastatin-hydroxylase activity and *in vivo* disposition of oral lovastatin (at 5 mg/kg).

As illustrated in Fig. 2, the loss of hepatic P450 activity in the LCN mouse leads to increased amounts of the un-metabolized drugs entering systemic circulation. At the same time, through mechanisms that may involve altered bile acid homeostasis and intestinal fibroblast growth factor 15 expression⁶⁸, intestinal P450 expression is upregulated, leading to increased intestinal metabolism of orally ingested drugs.

3.4. Genetic and epigenetic modifications of intestinal CYP expression or function

Genetic polymorphisms in various drug-metabolizing CYPs are well known⁶⁹ [<http://www.cypalleles.ki.se/>] and may lead to changes in drug-metabolizing activity in all organs that express a given P450 enzyme, including the intestine. The expression of CYP enzymes can also be regulated by epigenetic factors, such as microRNA (miRNA), which may have tissue-specific effects on the expression of a given gene^{70–72}. The general topic of the genetic polymorphisms and epigenetic regulations of various *CYP* genes have been reviewed recently⁷³. However, few studies have examined the impact of these factors on intestinal CYP expression.

3.5. The intestinal epithelium-specific *Cpr*-null (IECN) mouse as a model for studying consequences of suppressing intestinal P450 function

In IECN mouse, *Cpr* was deleted in intestinal enterocytes, leading to essential abolishment of all microsomal P450 activities in intestinal microsomes⁷⁴. CPR expression was normal in other tissues examined in IECN mice. These mice are fertile and develop normally, although they show hypersensitivity to intestinal injury induced by ricin, a plant-derived toxin⁷⁵, and dextran sulfate sodium, an agent used to induce colon inflammation in a commonly used animal model of experimental colitis⁷⁶. The IECN mouse also showed large changes in intestinal gene expression in cholesterol biosynthesis and antigen presentation/processing

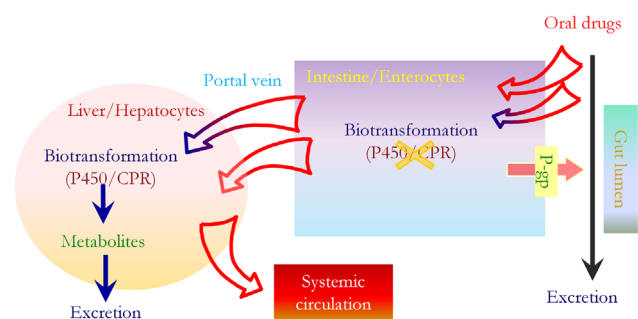


Figure 3 Impact of *Cpr* gene deletion in intestinal epithelial cells on first-pass metabolism of orally ingested drugs. Absorbed drugs cannot be metabolized by intestinal microsomal P450 enzymes in the IECN mouse, leading to increased amounts of the un-metabolized drugs arriving in the liver and systemic circulation.

pathways⁷⁷. The expression of most of the intestinal microsomal P450 enzymes was upregulated, but this did not lead to increased P450 activity given the absence of CPR. Hepatic P450 expression was not induced in the IECN mouse except for a slight induction of CYP1A1 and CYP1A2, the former was present at very low levels. The IECN mouse has been used to demonstrate the critical role of intestinal P450 enzymes in the *in vivo* metabolism and disposition of a number of xenobiotic compounds, including nifedipine, lovastatin, and midazolam^{66,67,74,78,79}. As depicted in Fig. 3, for these aforementioned compounds, the loss of intestinal P450 activity led to greater amounts of the un-metabolized parent compounds reaching systemic circulation. Notably, the finding that first-pass clearance of pravastatin, which is not noticeably metabolized by P450, was unchanged in the IECN mouse⁷⁹ confirmed that other events important for intestinal drug disposition, such as efflux transport, was unaffected by the loss of *Cpr* gene in the IECN model.

4. Assessment of the impact of intestinal drug metabolism on bioavailability

For orally administered drugs, the first-pass metabolism is contributed by both liver and small intestine⁸⁰ (Fig. 1). First-pass metabolism directly determines the bioavailability of oral drugs, which is expressed by the formula $F = F_A \times F_G \times F_H$ (F : bioavailability; F_A : fraction absorbed from intestinal lumen into enterocytes; F_G : fraction escaping intestinal metabolism and transferred to liver; F_H : fraction escaping hepatic metabolism and transferred to the systemic circulation)⁸¹. F_A , which is related to membrane permeability, has been extensively studied⁸²; it can be estimated by *in vitro* methods, such as a dissolution/permeation system^{83,84}. F_H can be derived from intrinsic hepatic clearance, blood flow rate, and protein binding⁸⁵. In contrast, F_G cannot be reliably assessed due to difficulties in separating intestinal and hepatic metabolism, which occurs in tandem *in vivo* (as illustrated in Fig. 1). Here, we will briefly review available methods and models for deriving F_G values from both clinical and preclinical data, compare F_G values calculated from different methods/models, and discuss possible utility of the IECN mouse model as a more accurate way to obtain F_G values. Notably, the ability to reliably assess F_G for oral drugs is critical for evaluating the impact of intestinal P450-mediated metabolism on oral bioavailability and to predict drug-drug interactions (DDI).

4.1. Estimation of F_G from clinical data

One practical way to estimate F_G is to conduct GFJ-drug interaction studies in patients⁸⁶. This approach is based on the following assumptions: (1) GFJ has no effect on F_A or F_H ; (2) GFJ can completely inhibit intestinal CYP3A4; and (3) the contribution of the intestine to systemic elimination of the drug under study is negligible. In this approach, the F_G values of 32 drugs, estimated from reported GFJ interaction studies, were found to range from 0.07 (for lovastatin) to 0.92 (for quinidine), indicating that, depending on the drug, the fraction of orally administered drugs eliminated by intestinal CYP3A4-mediated metabolism before reaching systemic circulation can range from 8% to as high as 93%⁸⁶.

Another way to assess F_G is based on comparisons between intravenous (i.v.) and oral dosing data⁸⁷. The oral bioavailability F can be obtained by comparing area under the concentration-time curve (AUC) of oral dosing to that of i.v. dosing. Thus, by calculating F_H as $F_H = 1 - CL_H/Q_H$ (CL_H : hepatic clearance; Q_H : average hepatic blood flow) and assuming $F_A = 1$ (complete absorption), one can obtain F_G by dividing F by F_H . With this method, F_G values of 21 drugs were estimated from reported clinical data⁸⁷.

Comparisons of the F_G estimates obtained for a collection of drugs using the two different methods showed good agreement for metabolized drugs that are not subject to transport, but not for drugs that are also substrates for P-gp and/or organic anion-transporting polypeptide transporters⁸⁶. The accuracy of these estimates could be affected by incomplete inhibition of intestinal CYP3A4 by GFJ, the choice of the Q_H value, which has a wide physiological range, and contribution of intestinal metabolism to systemic elimination, which is significant for some drugs⁸⁶.

A third method for assessing F_G , named the DDI method, was recently developed, which analyzes changes in pharmacokinetic properties caused by DDI⁸¹. The DDI method is based on the tissue-specific effect of a “perpetrator” drug on the $t_{1/2}$ of the victim drug, in that while inhibition of either liver or intestinal metabolism by the perpetrator results in an increase in the AUC, only inhibition of hepatic metabolism results in an increase in the $t_{1/2}$ of the victim drug. F_G values calculated using the DDI method showed good correlation ($r^2 = 0.81$) with those estimated using the GFJ method for CYP3A substrate drugs, but poor correlation ($r^2 = 0.41$) with those estimated using the i.v./oral method for a number of other drugs⁸¹.

The major features of these three methods are compared in Table 1. It is important to note that, despite the limitations and the sometimes differing F_G values obtained with each method, the results from the three methods all support the importance of intestinal P450-mediated metabolism for determining oral bioavailability for many drugs, even though the abundance of P450 enzymes in human intestine is much lower than that in the liver^{24,88}. The estimated role of intestinal P450 in reducing the bioavailability of a number of oral drugs is shown in Table 2^{81,86,89}, where the fraction eliminated by intestinal CYP3A during first-pass metabolism ranged from 8% (for quinidine) to 78% (for buspirone).

4.2. Estimation of F_G from preclinical data

A number of animal studies investigated the impact of first-pass metabolism on bioavailability of various oral drugs, by determining the effects of a CYP3A inhibitor (which often also inhibited P-gp)

Table 1 Comparison of F_G assessment methods based on clinical data.

Feature	GFJ	i.v./oral	DDI
Pros			
Provide estimation of F_G	×	$F_A \times F_G$	×
Incorporation of both intestinal and hepatic contribution	×		×
Only need pharmacokinetic data from oral dosing	×		×
Applicable to various P450 substrates		×	×
Cons			
Only for CYP3A substrates	×		
Require complete inhibition of intestinal metabolism	×		×
Inhibitors cannot affect hepatic metabolism	×		
Inhibitors cannot affect absorption/P-gp	×		×
Exact mechanism of inhibition is unknown	×		
Require pharmacokinetic data from i.v. dosing		×	
Fluctuate with choice of Q_H value		×	

Table 2 Estimated fractional elimination by intestinal metabolism and bioavailability of 15 orally administered CYP3A substrate drugs.

Drug	Bioavailability (F) ⁸⁹	Fraction eliminated by intestinal metabolism (%) ^a
Saquinavir	0.04	46.0
Buspirone	0.05	78.0
Nisoldipine	0.05–0.08	56.0
Atorvastatin	0.14	44.0
Felodipine	0.14	47.0
Verapamil	0.22	29.0
Cyclosporine	0.22–0.36	35.0–50.0
Midazolam	0.24–0.41	44.0–48.0
Sildenafil	0.38	18.0
Nifedipine	0.41	38.0
Alfentanil	0.42	39.0
Triazolam	0.55	36.0–45.50
Zolpidem	0.72	19.0
Quinidine	0.78	8.0
Alprazolam	0.84	11.0

^aFraction eliminated by intestinal metabolism was calculated by $(1 - F_G) \times 100$, where F_G was estimated from GFJ or DDI method^{81,86}.

on the pharmacokinetic parameters of a test drug^{90–97}. While these studies showed that first-pass metabolism played a role in limiting drug bioavailability, they did not identify the relative contributions of hepatic and intestinal metabolism, or of metabolism and efflux transport. For example, a study in rats demonstrated that the AUC and C_{max} values of orally dosed nifedipine were significantly increased, by >50%, in the presence of pioglitazone, a CYP3A4

inhibitor that also inhibits P-gp⁹⁷. The results from this study are insufficient for F_G calculation, but they do implicate P-gp-mediated transport in small intestine and CYP3A4-mediated metabolism in the small intestine and/or liver as potential contributors that affect the bioavailability of nifedipine.

Portal vein-cannulated rat represents a very useful surgical model for assessing intestinal disposition of drugs⁹⁸. This animal model allows simultaneous sampling of systemic and portal blood, which enables the estimation of $F_A \times F_G$ from the difference between portal and systemic blood concentrations after oral dosing in individual animals, without the need for i.v. drug administration. The $F_A \times F_G$ values determined for various drugs using this model were found to be relatively unaffected by changes in portal blood flow, in contrast to the fluctuation induced by changes in hepatic blood flow in the i.v./oral method⁹⁸. However, F_G cannot be separately assessed with this model. Moreover, variations in the surgical cannulation procedure may affect data consistency.

Considering the abundance of CYP3A in both liver and intestine and the involvement of CYP3A enzymes in the metabolism of >50% of the drugs in the clinic, the *Cyp3a*-knockout (*Cyp3a*^{-/-}) mice, and the hybrid *Cyp3a*^{-/-} mice that express human CYP3A4 in either liver hepatocytes (*Cyp3a*^{-/-}Tg-3A4_{hep}) or intestinal enterocytes (*Cyp3a*^{-/-}Tg-3A4_{int}) are highly useful for investigating the relative importance of intestinal versus hepatic CYP3A in first-pass metabolism⁹⁹. By comparing the pharmacokinetic parameters for the drug docetaxel among WT, *Cyp3a*^{-/-}, *Cyp3a*^{-/-}Tg-3A4_{int}, and *Cyp3a*^{-/-}Tg-3A4_{hep} models, it was clearly demonstrated that intestinal expression of CYP3A4 has a dominant effect on docetaxel oral bioavailability, whereas liver expression of CYP3A4 is the main contributor for systemic clearance⁹⁹. Similar findings were made with triazolam, another CYP3A substrate drug¹⁰⁰. Notably, a significant up-regulation of hepatic CYP2C expression in the *Cyp3a*-knockout mouse may complicate data interpretation for some drugs that are metabolized by both CYP3A and CYP2C, as in the case of midazolam¹⁰¹.

The IECN mouse model can provide direct quantitative data for the role of intestinal P450 in limiting oral drug bioavailability⁷⁴. The IECN model, which can be considered as an “optimized GFJ” model, has the following advantages over the GFJ model: (1) with abolishment of all microsomal P450 activities in the intestine, the IECN model is applicable to all P450 substrates; (2) there is no inhibition of hepatic metabolism according to the model characterization; (3) no change was observed in major intestinal drug transporters⁷⁷; and (4) the inhibition mechanism is well-defined and extent of inhibition is complete (gene knockout), which avoids variations related to GFJ brand, batch, and administration time seen in GFJ method.

Pharmacokinetic data derived from the WT and IECN mice can be used to calculate F_G . For examples, as shown in Table 3, based on the original data^{67,74,79}, the F_G values for nifedipine, lovastatin, and midazolam are found to be 0.31–0.69. The F_G of nifedipine estimated from the IECN model ($F_G=0.63$) is highly consistent with the value ($F_G=0.62$) calculated from clinical GFJ data⁸⁶. For midazolam and lovastatin, the F_G values estimated from IECN mice (0.69 and 0.31, respectively) are higher than the clinical GFJ data (0.56 and 0.07 for midazolam and lovastatin, respectively)⁸⁶ or the data from the DDI method (0.48 and 0.09 for midazolam and lovastatin, respectively)⁸¹. These discrepancies may be at least partly due to species differences. Notably, the F_G value of midazolam based on the IECN model (0.69) is comparable to the $F_A \times F_G$ value (0.71) estimated from the cannulated rat model⁹⁸.

Table 3 Pharmacokinetic parameters and F_G values estimated from IECN mouse model^a.

Drug	Strain	$t_{1/2}$ (h)	$AUC_{0-\infty}$ ^b	Estimation of F_G ^c
Nifedipine	WT	1.38 ± 0.74	8.0 ± 0.5	0.63
	IECN	0.91 ± 0.26	12.8 ± 2.3	
Lovastatin	WT	0.83 ± 0.15	23.5 ± 5.1	0.31
	IECN	1.13 ± 0.15*	76.4 ± 5.1	
Midazolam	WT	4.6 ± 0.5	5.8 ± 0.5	0.69
	IECN	2.9 ± 0.2**	8.4 ± 0.7	

^aThe pharmacokinetic parameters were taken from original publications for nifedipine⁷⁴, lovastatin⁷⁹, and midazolam⁶⁷ and they were determined after oral administration of the drugs at 10, 25, and 30 mg/kg, respectively.

^bThe units of $AUC_{0-\infty}$ for nifedipine, lovastatin, and midazolam were nmol · h/mL, $\mu\text{g} \cdot \text{min/mL}$, and nmol · h/mL, respectively.

^c F_G was calculated by $F_G = \frac{AUC(WT)}{AUC(IECN)}$.

* $P < 0.05$ compared to WT.

** $P < 0.01$ compared to WT.

For lovastatin, the oral bioavailability in mice is comparable to that in human (~5%)^{79,89}. Therefore, the higher F_G in mice suggests that the $F_A \times F_H$ in human is lower than in mice, indicating a better absorption and/or a less extensive hepatic first-pass metabolism of lovastatin in humans compared to mice. Similar scenario applies to midazolam, as the oral bioavailability in human (24%–41%)⁸⁹ is not lower than that in mice (21.5 ± 14.0%)¹⁰². In other words, human intestinal P450-mediated first-pass metabolism may play a greater role in determining oral bioavailability for some drugs than does the mouse counterpart.

5. Conclusions/perspectives

A large body of knowledge exists on the expression and regulation of intestinal P450 enzymes and their ability to metabolize various drugs and other xenobiotics, which can be incorporated into physiologically-based pharmacokinetic (PBPK) models to predict intestinal first-pass metabolism^{103–105}. A number of *in vivo* approaches have also been developed to more accurately determine the extent of intestinal P450-mediated metabolism of orally administered drugs. A combined utility of these models with other experimental models that target intestinal efflux transporters, such as P-gp, as illustrated for amprenavir and loperamide, which are substrates for both CYP3A4 and P-gp¹⁰⁶, would further identify potential interplay between intestinal P450-mediated metabolism and efflux transport, and distinguish relative contributions by the two related pathways.

Given the large number of drugs that are already known or predicted to subject to significant first-pass metabolism in the intestine by P450 enzymes, more studies are needed to identify patient-relevant pathophysiological factors that alter intestinal P450 expression and activity. Such knowledge would allow better prediction of disease-related changes or individualized variations in the bioavailability, and thus efficacy or safety, of many oral drugs. In addition, a better understanding of the mechanisms that underlie the recently discovered dietary regulation of intestinal P450⁶⁷ and the cross-talk between liver and intestine in the regulation of intestinal P450 expression⁶⁸ may lead to novel

strategies to modulate intestinal P450 expression in a clinical setting, in order to improve oral bioavailability for certain drugs.

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