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

Fang Xie, Xinxin Ding, Qing-Yu Zhang

Bioimaging, GlaxoSmithKline, King of Prussia, PA 19406, USA
College of Nanoscale Science, SUNY Polytechnic Institute, Albany, NY 12203 USA
Wadsworth Center, New York State Department of Health, Albany, NY 12201 USA

Received 28 April 2016; received in revised form 12 July 2016; accepted 14 July 2016

KEY WORDS
Cytochrome P450; Intestine; Bioavailability; Drug disposition; Drug metabolism

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.

© 2016 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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

*Corresponding author at: Wadsworth Center, New York State Department of Health, Empire State Plaza, Box 509, Albany, NY 12201-0509, USA. Tel.: +1 518 474 3728; fax: +1 518 473 8722.
E-mail address: qing-yu.zhang@health.ny.gov (Qing-Yu Zhang).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.07.012
2211-3835 © 2016 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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. 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. 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 detected. 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 P450s. 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 CYP2C19. An earlier study using metabolic activities to monitor the expression of different CYP2C isoforms in the human small intestine (dilofenac 4'-hydroxylase for CYP2C9 and mephentoin 4'-hydroxylase for CYP2C19), showed 17–18-fold differences for these CYPs among the intestines investigated.

Of the less abundant CYP enzymes in the intestine, CYP2J2 has been studied intensively. 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 tamoxifen.

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

Several studies have examined developmental expression of CYPs in the human intestine. The orphan P450 P4502W1 is expressed in fetal intestine, but its expression is suppressed soon after birth. 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 intestine. CYP3A4 is expressed in both prenatal and postnatal intestine; its expression level in neonatal duodenal tissue increased with age. 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 previously. 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. Many isoforms, including CYP1A1, 1B1, 1B9, 2B10, 2B19, 2C29, 2C38, 2C40, 2E1, 2J6, 3A11, 3A13, 3A16, 3A25, and 3A44, were identified, whereas several others, including CYP1A2, 2A, 2C7, 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 liver. 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 intestine.

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 6β-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 CYP4F30,31. 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 monkeys33,34,55. Several studies also characterized intestinal CYP expression in the common marmosets, another species of non-human primate35–37.

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 rats1,38–40, and dogs38,41,42. 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 subtype 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 pre-systemic 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 microsomes6,43, and the intestinal CYP3A activities towards representative CYP3A substrates were different by 2–5 folds between humans and rats1. 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 pyrazole35. 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 gut84. Mouse intestinal CYP1A1 and CYP2B10 were both induced by repeated oral administration of the antiparasitic drug ivermectin55. Mouse intestinal CYP3A expression was suppressed by insulin treatment46, although the effects of experimental models of type I and type II diabetes on intestinal CYP expression or activity seemed contradicting in two different studies46,75. 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 intestine86. Human CYP3A4 was induced by 3,3'-diindolylmethane, a herbal nutritional supplement, and piperine, a black pepper constituent, in human intestinal cells via PXR49,50. On the other hand, induction of CYP3A4 by 1α,25-dihydroxyvitamin D3 in Caco-2 cells was inhibited by androgrohalide, another herbal ingredient1. In rats, intestinal CYP3A1 expression was increased by the plasticizer acetyl tributyl citrate; the latter also increased CYP3A4 expression in human intestinal cells33. Intestinal expression of several rat CYPs, including CYP1A1, CYP2E1, and CYP3A9, was suppressed by treatment of rats with probiotic Lactobacillus casei53. 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 intestine25. In a rat model of indomethacin–induced intestinal ulcers, a small decrease in CYP2D2 expression was found in the upper part of the small intestine26.

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 drug\textsuperscript{57}. The metabolism of numerous drugs, including coumarin, cyclosporine, ethinylestradiol, midazolam, terfenadine, and verapamil\textsuperscript{58,59}, saquinavir\textsuperscript{60,61}, and erythromycin\textsuperscript{62}, 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 GFJ\textsuperscript{55}. Notably, orally ingested GFJ did not seem to affect hepatic CYP3A4 expression or activity, while decreasing small-intestinal CYP3A4 protein levels by \textgreater\textgreater 60\%\textsuperscript{64}. In contrast, the consumption of cranberry extract, which caused moderate anti-inflammatory drug, GFJ extract inhibited the \textit{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 patients\textsuperscript{66}.

Potential dietary regulation of intestinal drug metabolism is also illustrated by the effects of a synthetic diet on intestinal P450 expression and function\textsuperscript{67}. 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 \textit{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 \textit{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 \textit{Cpr}-null (LCN) mouse model, in which the activities of all microsomal P450s are suppressed in the hepatocytes due to deletion of the \textit{Cpr} gene\textsuperscript{68}. The loss of hepatocyte \textit{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 \textit{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 expression\textsuperscript{68}, 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 known\textsuperscript{69} [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\textsuperscript{70,71}. The general topic of the genetic polymorphisms and epigenetic regulations of various CYP genes have been reviewed recently\textsuperscript{73}. However, few studies have examined the impact of these factors on intestinal CYP expression.

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

In IECN mouse, \textit{Cpr} was deleted in intestinal enterocytes, leading to essential abolishment of all microsomal P450 activities in intestinal microsomes\textsuperscript{75}. 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 ricitin, a plant-derived toxin\textsuperscript{75}, and dextran sulfate sodium, an agent used to induce colon inflammation in a commonly used animal model of experimental colitis\textsuperscript{76}. The IECN mouse also showed large changes in intestinal gene expression in cholesterol biosynthesis and antigen presentation/processing
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 intestine\(^8^0\) (Fig. 1). First-pass metabolism directly determines the bioavailability of orally administered 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). \( F_A \), which is related to membrane permeability, has been extensively studied\(^8^2\); it can be estimated by in vitro methods, such as a dissolution/permeation system\(^8^3,8^4\). \( F_H \) can be derived from intrinsic hepatic clearance, blood flow rate, and protein binding\(^8^5\). 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 patients\(^8^6\). 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%\(^8^6\).

Another way to assess \( F_G \) is based on comparisons between intravenous (i.v.) and oral dosing data\(^8^7\). 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. By calculating \( F_H \) as \( F_H = 1 - CL_A / Q_H \) (\( CL_H \): 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 data\(^8^7\).

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 transporters\(^8^6\). 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 drugs\(^8^6\).

A third method for assessing \( F_G \), named the DDI method, was recently developed, which analyzes changes in pharmacokinetic properties caused by DDI\(^8^1\). 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 \( t_{1/2} \) 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 drugs\(^8^7\).

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\(^8^1,8^3,8^5\). The estimated role of intestinal P450 in reducing the bioavailability of a number of oral drugs is shown in Table 2\(^8^1,8^2,8^3,8^5\), where the fraction eliminated by intestinal CYP3A4 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)
studies showed that on the pharmacokinetic parameters of a test drug. 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 drugs. 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 method. 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$^{mice}$) or intestinal enterocytes (Cyp3a$^{-/-}$/Tg-3A4$^{int}$) are highly useful for investigating the relative importance of intestinal versus hepatic CYP3A in first-pass metabolism. By comparing the pharmacokinetic parameters for the drug docetaxel among WT, Cyp3a$^{-/-}$, Cyp3a$^{-/-}$/Tg-3A4$^{mice}$, and Cyp3a$^{-/-}$/Tg-3A4$^{int}$ 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 clearance. Similar findings were made with triazolam, another CYP3A substrate drug. 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 midazolam.

The IECN mouse model can provide direct quantitative data for the role of intestinal P450 in limiting oral drug bioavailability. 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 transporters; (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, the $F_G$ values for midazolam, 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 data. 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) or the data from the DDI method (0.48 and 0.09 for midazolam and lovastatin, respectively). 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 model.

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

| Feature                                      | GFJ i.v./oral DDI |
|----------------------------------------------|-------------------|
| Pros                                         | ×                 |
| Provide estimation of $F_G$                   | ×                 |
| 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 | ×                 |
| Flucluate with choice of $Q_{HI}$ value       | ×                 |

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

| Drug      | Bioavailability ($F$) | Fraction eliminated by intestinal metabolism (%) |
|-----------|-----------------------|-----------------------------------------------|
| 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                                          |

*Fraction eliminated by intestinal metabolism was calculated by $(1–F_G)\times100$, where $F_G$ was estimated from GFJ or DDI method.*
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-gp\(^{106}\), 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 pathophysiologic 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 P450\(^{107}\) and the cross-talk between liver and intestine in the regulation of intestinal P450 expression\(^{108}\) may lead to novel strategies to modulate intestinal P450 expression in a clinical setting, in order to improve oral bioavailability for certain drugs.

Acknowledgment

This work was supported in part by grants from the U. S. National Institutes of Health (CA092596, ES020867, and GM082978).

Table 3 Pharmacokinetic parameters and \(F_G\) values estimated from IECN mouse model.

| Drug     | Strain | \(t_{1/2}\) (h) | AUC\(_{0-\infty}\)\(^b\) | Estimation of \(F_G\) |
|----------|--------|----------------|-------------------------|----------------------|
| 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               |                      |

\(^a\)The pharmacokinetic parameters were taken from original publications for nifedipine\(^{7}\), lovastatin\(^{79}\), and midazolam\(^7\) and they were determined after oral administration of the drugs at 10, 25, and 30 mg/kg, respectively.

\(^b\)The units of AUC\(_{0-\infty}\) for nifedipine, lovastatin, and midazolam were nmol h/mL, \(\mu\)g min/mL, and nmol h/mL, respectively.

\(F_G\) was calculated by \(F_G = \frac{AUC_{IECN}}{AUC_{WT}}\).

\(^*P<0.05\) compared to WT.

\(^{**}P<0.01\) compared to WT.

References

1. Kaminsky LS, Zhang QY. The small intestine as a xenobiotic-metabolizing organ. Drug Metab Dispos 2003;31:1520–5.
2. Ding X, Kaminsky LS. Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. Annu Rev Pharmacol Toxicol 2003;43:149–73.
3. van Herwaarden AE, van Waterschoot RA, Schinkel AH. How important is intestinal cytochrome P450 3A metabolism? Trends Pharmacol Sci 2009;30:223–7.
4. Thelen K, Dressman JB. Cytochrome P450-mediated metabolism in the human gut wall. J Pharm Pharmacol 2009;61:541–58.
5. Zhang QY, Dunbar D, Ostrowska A, Zeisloft S, Yang J, Kaminsky LS. Characterization of human small intestinal cytochromes P-450. Drug Metab Dispos 1999;27:804–9.
6. Paine MF, Hart HL, Ludington SS, Haining RL, Rettie AE, Zeldin DC. The human intestinal cytochrome P450 "pie". Drug Metab Dispos 2006;34:880–6.
7. Obach RS, Zhang QY, Dunbar D, Kaminsky LS. Metabolic characterization of the major human small intestinal cytochrome p450s. Drug Metab Dispos 2001;29:347–52.
8. Zeldin DC, Foley J, Goldsworthy SM, Cook ME, Boyle JE, Ma J, et al. CYP2J subfamily cytochrome P450s in the gastrointestinal tract: expression, localization, and potential functional significance. Mol Pharmacol 1997;51:931–43.
9. Xu M, Ju W, Hao H, Wang G, Li P. Cytochrome P450 2J2: distribution, function, regulation, genetic polymorphisms and clinical significance. Drug Metab Rev 2013;45:311–52.
10. Lee CA, Neul D, Clouser-Roche A, Dalvie D, Wester MR, Jiang Y, et al. Identification of novel substrates for human cytochrome P450 2J2. Drug Metab Dispos 2010;38:347–56.
11. Rylander T, Neve EP, Ingelman-Sundberg M, Oscarson M. Identification and tissue distribution of the novel human intestinal cytochrome P450 2SI (CYP2SI). Biochim Biophys Acta 2001;1521:529–35.
12. Nishida CR, Lee M, de Montellano PR. Efficient hypoxic activation of the anticancer agent AQ4N by CYP2S1 and CYP2W1. Mol Pharmacol 2010;78:497–502.
13. Xiao Y, Shinkyo R, Guengerich FP. Cytochrome P450 2SI is reduced by NADPH-cytochrome P450 reductase. Drug Metab Dispos 2011;39:944–6.
14. Wang K, Guengerich FP. Bioactivation of fluorinated 2-aryl-benzo[b]thiazole antitumor molecules by human cytochrome P450s 1A1 and 2W1 and deactivation by cytochrome P450 2S1. Chem Res Toxicol 2012;25:1740–51.
15. Choong E, Guo J, Persson A, Virding S, Johansson I, Mkrtchian S, et al. Developmental regulation and induction of cytochrome P450 2W1, an enzyme expressed in colon tumors. PLoS One 2015;10:e0122820.
16. Chen YT, Trzoss L, Yang D, Yan B. Ontogenic expression of human carboxylesterase-2 and cytochrome P450 3A4 in liver and duodenum/postnatal surge and organ-dependent regulation. Toxicology 2015;330:55–61.
17. Betts S, Björkhem-Bergman L, Rane A, Ekström L. Expression of CYP3A4 and CYP3A7 in human foetal tissues and its correlation with nuclear receptors. Basic Clin Pharmacol Toxicol 2015;117:261–6.
18. Cizkova K, Konieczna A, Erdosova B, Ehrmann J. Time-dependent expression of cytochrome p450 epoxygenases during human prenatal development. Organogenesis 2014;10:53–61.
Intestinal P450 and drug disposition

19. Törönen R, Kärenlampi S, Pelkonen K. Hepa-1 enzyme induction assay as an in vitro indicator of the CYP1A1-inducing potencies of laboratory rodent diets in vivo. *Life Sci* 1994;55:1945–54.

20. Cunnings DA, Schut HA. Inhibitory effect of dietary 4-ipomeanol on DNA adduction by the food mutagen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in male CDF, mice. *Carcinogenesis* 1995;16:2523–9.

21. Muto N, Hirai H, Tanaka T, Itoh N, Tanaka K. Induction and inhibition of cytochrome P450 isoforms by imazalil, a food contaminant, in mouse small intestine and liver. *Xenobiotica* 1997;27:1215–23.

22. Emoto C, Yamazaki H, Yamasaki S, Shimada N, Nakajima M, Yokoi T. Characterization of cytochrome P450 enzymes involved in drug oxidations in mouse intestinal microsomes. *Xenobiotica* 2000;30:943–53.

23. Xie Q, Zhang QY, Zhang Y, Su T, Gu J, Kaminsky LS, et al. Induction of mouse CYP2J by pyrazole in the eye, kidney, liver, lung, olfactory mucosa, and small intestine, but not in the heart. *Drug Metab Dispos* 2000;28:1311–2006.

24. Zhang QY, Dunbar D, Kaminsky LS. Characterization of mouse small intestinal cytochrome P450 450 expression. *Drug Metab Dispos* 2003;31:1346–51.

25. Uno S, Dragan N, Miller ML, Dalton TP, Gonzalez FJ, Nebert DW, Basal and inducible CYP1 mRNA quantitation and protein localization throughout the mouse gastrointestinal tract. *Free Radic Biol Med* 2008;44:570–83.

26. Renaud HJ, Cui JY, Khan M, Klaassen CD. Tissue distribution and gender-divergent expression of 78 cytochrome P450 mRNAs in mice. *Toxicol Sci* 2011;124:261–77.

27. Hersman EM, Bumpus NN. A targeted proteomics approach for profiling murine cytochrome P450 expression. *J Pharmacol Exp Ther* 2014;349:221–31.

28. Nakaniishi Y, Matsuura A, Matsuno K, Iwasaki K, Uttoh M, Nakamura C, et al. Regional distribution of cytochrome p450 mRNA expression in the liver and small intestine of cynomolgus monkeys. *Drug Metab Pharmacokinet* 2010;25:290–7.

29. Nakaniishi Y, Matsuura A, Matsuno K, Iwasaki K, Uttoh M, Nakamura C, et al. Regional distribution of drug-metabolizing enzyme activities in the liver and small intestine of cynomolgus monkeys. *Drug Metab Pharmacokinet* 2011;26:288–94.

30. Nishimuta H, Nakagawa T, Nomura N, Yabuki M. Species differences in intestinal metabolic activities of cytochrome P450 isoforms between cynomolgus monkeys and humans. *Drug Metab Pharmacokinet* 2011;26:300–6.

31. Komura H, Iwaki M. In vitro and in vivo small intestinal metabolism of CYP3A substrates and UGT substrates in preclinical animals: species and human differences. *Drug Metab Rev* 2011;43:476–98.

32. Yoda N, Emoto C, Date S, Kondo S, Miyake M, Nakazato S, et al. Characterization of intestinal and hepatic P450 enzymes in cytochrome P450 enzymes with typical substrates and inhibitors for human P450 enzymes. *Xenobiotica* 2012;42:719–30.

33. Uehara S, Murayama N, Nakaniishi Y, Nakamura C, Hashizume T, Zeldin DC, et al. Immunohemchemical detection of cytochrome P450 enzymes in small intestine microsomes of male and female untreated juvenile cynomolgus monkeys. *Xenobiotica* 2014;44:769–74.

34. Uehara S, Murayama N, Nakaniishi Y, Nakamura C, Hashizume T, Zeldin DC, et al. Immunohemchemical quantification of cytochrome CYP3A4 and CYP3A7 enzymes in liver and small intestine. *Xenobiotica* 2015;45:124–30.

35. Uehara S, Uno Y, Inoue T, Sasaki E, Yamazaki H. Molecular cloning, tissue distribution, and functional characterization of marmoset cytochrome P450 450 450 in livers and small intestines metabolizes typical human P450 2D6 substrates, metoprolol, bufuralol and dextromethorphan. *Xenobiotica* 2015;45:766–72.

36. Shimizu M, Iwano S, Uno Y, Uehara S, Inoue T, Murayama N, et al. Qualitative de novo analysis of full length cDNA and quantitative analysis of gene expression for common marmoset (Cattilinus jucuus) transcriptomes using parallel long-read technology and short-read sequencing. *PLoS One* 2014;9:e100036.

37. Nishimuta H, Nakagawa T, Nomura N, Yabuki M. Species differences in hepatic and intestinal metabolic activities for 43 human cytochrome P450 substrates between humans and rats or dogs. *Xenobiotica* 2013;43:948–55.

38. Palasz A, Wiaderkiewicz A, Wiaderkiewicz R, Czajk F, Czajkowski B, Lebda-Wyborny T, et al. Age-related changes in the mRNA levels of CYP1A1, CYP2B1, and CYP3A1 isoforms in rat small intestine. *Gene* 2011;27:197–207.

39. Bueters T, Juric S, S oluens-Stembeck AK, Hu Y, Bylund J. Rat poorly predicts the combined non-absorbed and presystemically metabolized fractions in the human. *Xenobiotica* 2013;43:607–16.

40. Heikkilä AT, Friedlein A, Matondo M, Hatley OJ, Petsalo A, Juvenon R, et al. Quantitative ADME proteomics—CYP and UGT enzymes in the Beagle dog liver and intestine. *Pharm Res* 2015;32:74–90.

41. Heikkilä AT, Fowler S, Gray L, Li J, Peng Y, Yadava P, et al. In vitro to in vivo extrapolation and physiologically based modeling of cytochrome P450 mediated metabolism in beagle dog gut wall and liver. *Mol Pharm* 2013;10:1388–99.

42. Matsubara T, Kim HJ, Miyata M, Shimada M, Nagata K, Yamaoze Y. Isolation and characterization of a new major intestinal CYP3A form, CYP3A62, in the rat. *J Pharmacol Exp Ther* 2004;309:1282–90.

43. Do KN, Fink LN, Jensen TE, Gaultier L, Parlesak A. TLR2 controls intestinal carcinogen detoxification by CYP1A1. *PLoS One* 2012;7:e32309.

44. Alberich M, Menez C, Sutra JF, Lepine A. Ivermectin exposure leads to up-regulation of detoxification genes in vitro and in vivo in mice. *Eur J Pharmacol* 2014;740:428–35.

45. Kudo T, Toda T, Ushiki T, Ohk I, Ikarashi N, Ochiai W, et al. Differences in the pharmacokinetics of CYP3A substrates in TSOD and streptozotocin-induced diabetic mice. *Xenobiotica* 2010;40:282–90.

46. Patoine D, Petit M, Pilote S, Picard F, Drolet B, Simard C. Modulation of CYP3A expression and activity in mice models of type 1 and type 2 diabetes. *Pharmacol Res Perspect* 2014;2:e00082.

47. Boušová I, Matoušková P, Bártíková H, Szotáková B, Hautsova V, Tománková V, et al. Influence of diet supplementation with green tea extract on drug-metabolizing enzymes in a mouse model of monosodium glutamate-induced obesity. *Eur J Nutr* 2016;55:361–71.

48. Pardugula SR, Flannery PC, Abbott KL, Coleman ES, Mani S, et al. Diidolymethane, a naturally occurring compound, induces CYP3A4 and MDR1 gene expression by activating human PXR. *Toxicol Lett* 2015;232:580–9.

49. Wang YM, Lin W, Chai SC, Wu J, Ong SS, Schuetz EG, et al. Piperine activates human pregnane X receptor to induce the expression of cytochrome P450 3A4 and multidrug resistance protein 1. *Toxicol Appl Pharmacol* 2013;272:96–107.

50. Qi F, Hou XL, Takahashi K, Chen LX, Azuma J, Kang A. Androgapholide inhibits the expression and metabolic activity of cytochrome P450 3A4 in the modified Caco-2 cells. *J Ethnopharmacol* 2012;141:709–13.

51. Takeshita A, Igarashi-Migitaka J, Nishiyama K, Takahashi H, Takeuchi Y, Koibuchi N. Acetyl tributyl citrate, the most widely used phthalate substitute plasticizer, induces cytochrome P450 3A4 through steroid and xenobiotic receptor. *Toxicol Sci* 2011;123:469–70.

52. Matuskova Z, Siller M, Tunkova A, Anzenbacherova E, Zacharova A, Tlaskalova-Hogenova H, et al. Effects of Lactobacillus casei on the expression and the activity of cytochromes P450 and on the CYP mRNA level in the intestine and the liver of male rats. *Neuroendo-criol Lett* 2011;32:Suppl I:18–14.

53. Matuskova Z, Tunkova A, Anzenbacherova E, Vecera R, Siller M, Tlaskalova-Hogenova H, et al. Effects of probiotic Esherichia coli
Nissel 1917 on expression of cytochromes P450 along the gastrointestinal tract of male rats. Neuroendocrinol Lett 2010;31(Suppl):246–50.

55. Kawauchi S, Nakamura T, Miki I, Inoue J, Hamaguchi T, Tanahashi T, et al. Downregulation of CYP3A and P-glycoprotein in the secondary inflammatory response of mice with dextran sulfate sodium-induced colitis and its contribution to cyclosporine A blood concentrations. J Pharmacol Sci 2014;124:180–91.

56. Kawauchi S, Nakamura T, Yasui H, Nishikawa C, Miki I, Inoue J, et al. Intestinal and hepatic expression of cytochrome P450s and MDR1A in rats with indomethacin-induced small intestinal ulcers. Int J Med Sci 2014;11:1208–17.

57. Bailey DG, Malcolm J, Arnold O, Spence JD. Grapefruit juice-drug interactions. Br J Clin Pharmacol 1998;46:101–10.

58. Ameer B, Weintrab R A. Drug interactions with grapefruit juice. Clin Pharmacokinet 1997;33:103–21.

59. Fuhr U. Drug interactions with grapefruit juice. Extent, probable mechanism and clinical relevance. Drug Saf 1998;18:251–72.

60. Kupferschmidt HH, Fattinger KE, Ha HR, Follath F, Krähenbühl S. Grapefruit juice enhances the bioavailability of the HIV protease inhibitor saquinavir in man. Br J Clin Pharmacol 1998;45:355–9.

61. Eagling VA, Proctor CE, Lown KS, Bailey DG, Fortlage D, Janardan SK, et al. Grapefruit juice-drug interactions. Br J Clin Pharmacol 1998;46:101–10.

62. Kanazawa S, Ohkubo T, Sugawara K. The effects of grapefruit juice on the human cytochrome P450 3A activity. Nutr Res 1998;20:85–91.

63. Guo LQ, Taniguchi M, Xiao YQ, Baba K, Ohta T, Yamazoe Y. Inhibitory effect of natural furanocoumarins on human microsomal cytochrome P450 3A activity. Jpn J Pharmacol 2000;82:122–9.

64. Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, et al. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. J Clin Invest 1997;99:2545–53.

65. Bártiková H, Bousková I, Jedličková P, Lčněnková K, Skůlová L, Szotáková B. Effect of standardized cranberry extract on the activity of P-glycoprotein and CYP3A4 inhibition by simvastatin. J Clin Pharmacol 2001;41:362–6.

66. Guo LQ, Taniguchi M, Xiao YQ, Baba K, Ohta T, Yamazoe Y. Inhibitory effect of natural furanocoumarins on human microsomal cytochrome P450 3A activity. J Pharmacol Exp Ther 2001;294:362–70.

67. Zhang P, Jia K, Fang C, Zhou X, Ding X, Zhang QY. Dietary regulation of mouse intestinal P450 expression and drug metabolism. Drug Metab Dispos 2013;41:529–35.

68. Zhu Y, Ding X, Fang C, Zhang QY. Regulation of intestinal drug expression by hepatic cytochrome P450 3A4: possible involvement of fibroblast growth factor 15 and impact on systemic drug exposure. Mol Pharmacol 2014;85:139–47.

69. Sim SC, Ingelman-Sundberg M. The Human Cytochrome P450 (CYP) Allele Nomenclature website: a peer-reviewed database of CYP variants and their associated effects. Hum Genom 2010;4:278–81.

70. Gomez A, Ingelman-Sundberg M. Epigenetic and microRNA-dependent control of cytochrome P450 expression: a gap between DNA and protein. Pharmacogenomics 2009;10:1067–76.

71. Tamási V, Monostory K, Prough RA, Falus A. Role of xenobiotic metabolism in cancer: involvement of transcriptional and miRNA regulation of P450s. Cell Mol Life Sci 2011;68:1131–46.

72. Ikemura K, Iwamoto T, Okada M. MicroRNAs as regulators of drug transporters, drug-metabolizing enzymes, and tight junctions: implications for interindividual differences in drug disposition. Curr Top Pharmacol 2014;43:217–34.

73. Zanger UM, Klein K, Thomas M, Rieger JK, Tremmel R, Kandel BA, et al. Genetics, epigenetics, and regulation of drug-metabolizing cytochrome P450 enzymes. Clin Pharmacol Ther 2014;95:258–61.

74. Zhang QY, Fang C, Zhang J, Dunbar D, Kaminsky L, Ding X. An intestinal epithelium-specific cytochrome P450 (P450) reductase-knockout mouse model: direct evidence for a role of intestinal P450s in first-pass clearance of oral nifedipine. Drug Metab Dispos 2009;37:651–7.

75. Ahlawat S, Xie F, Zhu Y, D’Hondt R, Ding X, Zhang QY, et al. Mice deficient in intestinal epithelium cytochrome P450 reductase are prone to acute toxin-induced mucosal damage. Sci Rep 2014;4:5551.

76. Zhu Y, Xie F, Ding L, Fan X, Ding X, Zhang QY. Intestinal epithelium-specific knockout of the cytochrome P450 reductase gene exacerbates dextran sulfate sodium-induced colitis. J Pharmacol Exp Ther 2015;354:10–7.

77. D’Agostino J, Ding X, Zhang P, Jia K, Fang C, Zhu Y, et al. Potential biological functions of cytochrome P450 reductase-dependent enzymes in small intestine: novel link to expression of major histocompatibility complex class II genes. J Biol Chem 2012;287:17777–88.

78. Fang C, Zhang QY. The role of small-intestinal P450 enzymes in protection against systemic exposure of orally administered benzo[a]pyrene. J Pharmacol Exp Ther 2010;334:156–63.

79. Zhu Y, D’Agostino J, Zhang QY. Role of intestinal cytochrome P450 (P450) in modulating the bioavailability of oral lovastatin: insights from studies on the intestinal epithelium-specific P450 reductase knockout mouse. Drug Metab Dispos 2011;39:939–43.

80. Doherty MM, Charman WN. The mucosa of the small intestine: how clinically relevant as an organ of drug metabolism? Clin Pharmacokinet 2002;41:235–53.

81. Hisaka A, Nakamura M, Tsukihashi A, Koh S, Suzuki H. Assessment of intestinal availability (Ffi) of substrate drugs of cytochrome p450s by analyzing changes in pharmacokinetic properties caused by drug-drug interactions. Drug Metab Dispos 2014;42:1640–5.

82. Amidon GL, Sinko PJ, Fleisher D. Estimating human oral fraction dose absorbed: a correlation using rat intestinal membrane permeability for passive and carrier-mediated compounds. Pharm Res 1988;5:651–4.

83. Kansy M, Senner F, Gubker B. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the design of passive absorption processes. J Med Chem 1998;41:1007–10.

84. Miyaji Y, Fujiy I, Takeyama S, Kawai Y, Kataoka M, Takahashi M, et al. Advantage of the dissolution/permeation system for estimating oral absorption of drug candidates in the drug discovery stage. Mol Pharm 2016;13:1564–74.

85. Chiba M, Ishii Y, Sugiyama Y. Prediction of hepatic clearance in human from in vitro data for successful drug development. AAPS J 2009;11:262–76.

86. Gertz M, Davis JD, Harrison A, Houston JB, Galetin A. Grapefruit juice-drug interaction studies as a method to assess the extent of intestinal availability: utility and limitations. Curr Drug Metab 2008;9:785–95.

87. Galetin A, Gertz M, Houston JB. Potential role of intestinal first-pass metabolism in the prediction of drug–drug interactions. Expert Opin Drug Metab Toxicol 2008;4:909–22.

88. Paine MF, Khaliq M, Fisher JM, Shen DD, Kunze KL, Marsh CL, et al. Characterization of interintestinal and interintestinal variations in human CYP3A-dependent metabolism. J Pharmacol Exp Ther 1997;283:1552–62.

89. Galetin A, Gertz M, Houston JB. Contribution of intestinal cytochrome P450-mediated metabolism to drug–drug inhibition and induction interactions. Drug Metab Pharmacokinet 2010;25:28–47.

90. Choi DH, Li C, Choi JS. Effects of myricetin, an antioxidant, on the pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats: possible role of cytochrome P450 3A4, cytochrome P450 2C9 and P-glycoprotein inhibition by myricetin. J Pharmacol Pharmacol 2010;62:908–14.

91. Choi DH, Chung JH, Choi JS. Pharmacokinetic interaction between oral lovastatin and verapamil in healthy subjects: role of P-glycoprotein inhibition by lovastatin. Eur J Clin Pharmacol 2010;66:285–90.

92. Choi DH, Choi JS, Li C, Choi JS. Effects of simvastatin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, after oral and intravenous administration in rats: possible role of P-glycoprotein and CYP3A4 inhibition by simvastatin. Pharmacol Rep 2011;63:1574–82.
93. Cho YA, Choi JS, Burm JP. Effects of the antioxidant baicalein on the pharmacokinetics of nimodipine in rats: a possible role of P-glycoprotein and CYP3A4 inhibition by baicalein. *Pharmacol Rep* 2011;63:1066–73.

94. Cho YA, Lee W, Choi JS. Effects of curcumin on the pharmacokinetics of tamoxifen and its active metabolite, 4-hydroxytamoxifen, in rats: possible role of CYP3A4 and P-glycoprotein inhibition by curcumin. *Pharmazie* 2012;67:124–30.

95. Chen Y, Zhang W, Li D, Ai J, Meng Y, Ying X, et al. Hepatic and gastrointestinal first-pass effects of vitexin-4’-O-glucoside in rats. *J Pharm Pharmacol* 2013;65:1500–7.

96. Choi JS, Choi I, Choi DH. Effects of nifedipine on the pharmacokinetics of repaglinide in rats: possible role of CYP3A4 and P-glycoprotein inhibition by nifedipine. *Pharmacol Rep* 2013;65:1422–30.

97. Choi JS, Choi I, Choi DH. Effects of pioglitazone on the pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats. *Eur J Drug Metab Pharmacokinet* 2016;41:231–8.

98. Matsuda Y, Konno Y, Satsukawa M, Kobayashi T, Takimoto Y, Morisaki K, et al. Assessment of intestinal availability of various drugs in the oral absorption process using portal vein-cannulated rats. *Drug Metab Dispos* 2012;40:2231–8.

99. van Herwaarden AE, Wagenaar E, van der Kruijssen CM, van Waterschoot RA, Song JY, Song JW, et al. Knockout of cytochrome P450 3A yields new mouse models for understanding xenobiotic metabolism. *J Clin Investig* 2007;117:3583–92.

100. van Waterschoot RA, Rooswinkel RW, Sparidans RW, van Herwaarden AE, Beijnen JH, Schinkel AH. Inhibition and stimulation of intestinal and hepatic CYP3A activity: studies in humanized CYP3A4 transgenic mice using triazolam. *Drug Metab Dispos* 2009;37:2305–13.

101. van Waterschoot RA, van Herwaarden AE, Lagas JS, Sparidans RW, Wagenaar E, van der Kruijssen CM, et al. Midazolam metabolism in cytochrome P450 3A knockout mice can be attributed to up-regulated CYP2C enzymes. *Mol Pharmacol* 2008;73:1029–36.

102. van Waterschoot RA, Rooswinkel RW, Sparidans RW, van Herwaarden AE, Wagenaar E, Beijnen JH, et al. Expression of the human CYP3A4 gene in the small intestine of transgenic mice: in vitro metabolism and pharmacokinetics of midazolam. *Drug Metab Dispos* 2003;31:548–58.

103. van Waterschoot RA, van Herwaarden AE, Lagas JS, Sparidans RW, Wagenaar E, Beijnen JH, et al. Assessment of intestinal availability of various drugs in the oral absorption process using portal vein-cannulated rats. *Drug Metab Dispos* 2012;40:2231–8.

104. van Waterschoot RA, van Herwaarden AE, Lagas JS, Sparidans RW, Wagenaar E, Beijnen JH, et al. Midazolam metabolism in cytochrome P450 3A knockout mice can be attributed to up-regulated CYP2C enzymes. *Mol Pharmacol* 2008;73:1029–36.

105. van Waterschoot RA, van Herwaarden AE, Lagas JS, Sparidans RW, Wagenaar E, Beijnen JH, et al. Expression of the human CYP3A4 gene in the small intestine of transgenic mice: in vitro metabolism and pharmacokinetics of midazolam. *Drug Metab Dispos* 2003;31:548–58.

106. van Waterschoot RA, Rooswinkel RW, Sparidans RW, van Herwaarden AE, Beijnen JH, Schinkel AH. Inhibition and stimulation of intestinal and hepatic CYP3A activity: studies in humanized CYP3A4 transgenic mice using triazolam. *Drug Metab Dispos* 2009;37:2305–13.