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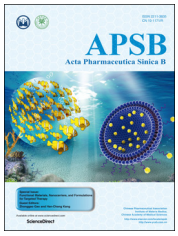

REVIEW

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

Fang Xie[a], **Xinxin Ding**[b], **Qing-Yu Zhang**[c,*]

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

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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.

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).

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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 detected[5]. 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[6]. 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[6]. 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 investigated[7].

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 tamoxifen[10].

Another P450 with a somewhat preferential expression in the intestine is CYP2S1[11]. 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-fluorobenzothiazole[14].

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 birth[15]. 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[18]. 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 previously[1]. 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 liver[26]. 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

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