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DRUG METABOLISM & DISPOSITION

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In Vitro Human Cell–Based Experimental Models for the Evaluation of Enteric Metabolism and Drug Interaction Potential of Drugs and Natural Products

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ABSTRACT

Elements of key enteric drug metabolism and disposition pathways are reviewed to aid the assessment of the applicability of current cell-based enteric experimental systems for the evaluation of enteric metabolism and drug interaction potential. Enteric nuclear receptors include vitamin D receptor, constitutive androstane receptor, pregnane X receptor, farnesoid X receptor, liver X receptor, aryl hydrocarbon receptor, and peroxisome proliferator–activated receptor. Enteric drug metabolizing enzyme pathways include both cytochrome P450 (P450) and non-P450 drug metabolizing enzymes based on gene expression, proteomics, and activity. Both uptake and efflux transporters are present in the small intestine, with P-glycoprotein found to be responsible for most drug-drug and food-drug interactions. The cell-based in vitro enteric systems reviewed are 1) immortalized cell line model: the human colon adenocarcinoma (Caco-2) cells; 2) human stem cell–derived enterocyte models: stem cell enteric systems, either from intestinal crypt cells or induced pluripotent stem cells; and 3) primary cell models: human intestinal slices, cryopreserved human enterocytes, permeabilized cofactor-supplemented (MetMax) cryopreserved human enterocytes, and cryopreserved human intestinal mucosa. The major deficiency with both immortalized cell lines and stem cell–derived enterocytes is that drug metabolizing enzyme activities, although they are detectable, are substantially lower than those for the intestinal mucosa in vivo. Human intestine slices, cryopreserved human enterocytes, MetMax cryopreserved human enterocytes, and cryopreserved human intestinal mucosa retain robust enteric drug metabolizing enzyme activity and represent appropriate models for the evaluation of metabolism and metabolism-dependent drug interaction potential of orally administered xenobiotics including drugs, botanical products, and dietary supplements.

SIGNIFICANCE STATEMENT

Enteric drug metabolism plays an important role in the bioavailability and metabolic fate of orally administered drugs as well as in enteric drug-drug and food-drug interactions. The current status of key enteric drug metabolism and disposition pathways and in vitro human cell–based enteric experimental systems for the evaluation of the metabolism and drug interaction potential of orally administered substances is reviewed.

Introduction

Due to species differences, human-based in vitro experimental systems serve important functions in the assessment of human drug properties such as bioavailability, metabolic fate, drug-drug interaction potential, toxic potential, and pharmacological activity. For drug metabolism, emphasis routinely has been placed on hepatic events, with human hepatocytes serving as the "gold standard" due to the presence of all organelles and their associated drug metabolizing enzymes. Successful cryopreservation of human hepatocytes allows this experimental system to be routinely applied in drug development to guide the selection of the drug candidates for clinical trials, as well as in mechanistic research to further our knowledge in hepatic biology, including metabolism, toxicology, pharmacology, and gene/protein functions.

Human small intestines can arguably be as important as the liver as a determinant of human drug properties. Oral dosing is the preferred and predominant route of administration for drugs, herbal medicines, and health supplements. The small intestines serve as the gateway for the entrance of the orally administered xenobiotics into the systemic circulation via the provision of a biologic barrier, uptake and efflux transport, and metabolic clearance. In vitro human enteric models, akin to hepatocytes for the liver, are now being recognized as

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ABBREVIATIONS: Ahr, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; CHIM, cryopreserved human intestinal mucosa; FXR, farnesoid X receptor; GFJ, grapefruit juice; iPSC, induced pluripotent stem cell; LXR, liver X receptor; MAO, monoamine oxidase; MRP, multidrug resistance protein; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein; P450, cytochrome P450; PPAR, peroxisome proliferator–activated receptor; PXR, pregnane X receptor; UGT, UDP-glucuronosyltransferase; VDR, vitamin D receptor.

TABLE 1

Nuclear receptors identified in the human small intestine

Examples of ligands and effects of ligand binding on drug metabolizing enzyme and drug transporter expression are presented.

Nuclear receptors	Ligands	Effects of activation on enteric drug metabolism enzyme targets	Effects of activation on enteric transporter targets
VDR	1,25(OH)2D3	Induction of CYP24A1, CYP3A4	Induction of SLC30A10 (zinc and manganese transporter ZnT10), ABCB1 (P-glycoprotein), proton-coupled folate transporter, apical sodium-dependent bile acid transporter
CAR	Phenobarbital; phenytoin; polybrominated biphenyls	Induction of CYP2B6, CYP3A4/5/7	Induction of ABCB1 (P-glycoprotein),
PXR	Rifampin, phenobarbital, statins, and St. John's wort	Induction of CYP3A4/5/7, CYP27A1	Induction of ABCB1 (P-glycoprotein)
FXR	Bile salts	Induction of intestinal fibroblast growth factor 19, which activates hepatic fibroblast growth factor receptor 4, resulting in repression of hepatic CYP7A	Induction of SLC51A and B (OSTα and OSTβ)
LXR	Oxysterols	Unknown for enteric drug metabolizing enzymes (Induction of hepatic CYP7A1)	Induction ABCG5/ABCG8 (transporters responsible for cholesterol efflux)
AhR	Aryl hydrocarbons (3-methylcholanthrene; b-naphthoflavone); TCDD	Induction of CYP1A1, CYP1A2, UGT	Not known
PPAR-β/δ	Fatty acids and fatty-acid metabolites	Not known	Not known

TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

important tools to provide information complementing that obtained with in vitro hepatic models to improve our understanding of the properties of orally administered drugs. As a large and well perfused organ, the small intestine may also contribute extensively to xenobiotic metabolism in the systemic circulation.

This review will focus on current advances in human cell–based experimental systems for the assessment of the metabolic fate of orally administered xenobiotics. The strengths and limitations of the available in vitro experimental systems to model key enteric events will be reviewed, with an emphasis on their applicability for the evaluation of drug metabolism and drug interaction potential of orally administered drugs and natural products. Nonhuman systems and cell-free experimental models such as enteric microsomes are not included. This review is intended to be complementary to that recently reported by Sawant-Basak et al. (2018), with a focus on enteric drug metabolism, which is not extensively covered in other reviews of the human small intestine (Gjorevski and Ordonez-Moran, 2017; Pearce et al., 2018; Dutton et al., 2019; Ponce de León-Rodríguez et al., 2019; Maares and Haase, 2020).

Clinical Significance of Enteric Drug Metabolism

The significance of enteric metabolism in drug disposition is illustrated by the accidental discovery of the effects of grapefruit juice (GFJ) intake on oral drug bioavailability of the dihydropyridine calcium channel antagonist, felodipine. Patients taking the drug with GFJ had plasma concentrations several-fold higher than expected, an event eventually attributed to the inhibitory effects of GFJ on presystemic enteric metabolism of the drug (Bailey et al., 1989; Lown et al., 1997; Gandhi et al., 2013). This clinically significant phenomenon of GFJ-drug interaction subsequently was extended to a long list of orally administered drugs that are substrates of CYP3A (Bailey et al., 1998), the cytochrome P450 (P450) isoform known to be responsible for the hepatic metabolism of over 50% of known drugs (Li et al., 1995). Clinical evaluation of the effects of GFJ upon intravenous and oral administration of the CYP3A4 probe substrate, midazolam, demonstrates conclusively that enteric drug metabolism, not hepatic metabolism, is responsible for this phenomenon (Kupferschmidt et al., 1995). The GFJ phenomenon highlights two important aspects of enteric drug metabolism: 1) enteric drug metabolism can be a major determinant of oral bioavailability and subsequent plasma drug concentrations (Mizuma, 2002; Galetin et al., 2008; Chen and Yu, 2009), and 2) bioavailability of a drug can be significantly affected by enteric contents, including coadministered drugs, foods, and dietary supplements, that can have a significant impact on the activity of enteric drug metabolizing enzymes (Won et al., 2010). It is to be noted that, beside CYP3A4 inhibition, GFJ is known to cause clinically significant drug interactions via its inhibitory effects on drug transporters including the elevation of plasma drug concentrations via inhibiting P-glycoprotein (P-gp)–mediated efflux (Bailey and Dresser, 2004) and the reduction of plasma drug concentrations via inhibition of organic anion transporting polypeptide (OATP)–mediated drug uptake (Dresser et al., 2005).

Enteric Nuclear Receptors

Identification of enteric nuclear receptors is key to the understanding drug metabolizing enzyme gene expression, which can provide insight on environmental and genetic effects on enteric drug metabolism. The key enteric nuclear receptors with regulatory roles in drug metabolizing enzyme expression are presented in Table 1 and described as follows:

1. Vitamin D receptor (VDR): VDR is a unique enteric receptor originally detected in nuclei of enterocytes lining crypts in duodenal mucosa (Colston et al., 1994), and later found all through the human small intestine and colon (Barbáchano et al., 2017) as well as other extrahepatic organs including bone, kidney, parathyroid glands, and tumors (Pike et al., 2017). VDR binding by the active vitamin D metabolite, 1alpha,25-dihydroxyvitamin D3 (D3), leads to increased expression of CYP24A1, which is responsible for its catabolism (Peng et al., 2012; Li et al., 2018a), as well as the induction of CYP3A4, which may lead to increased presystemic metabolism of orally administered drugs (Makishima et al., 2002; Thompson et al., 2002; Li et al., 2018a). D3 binding to VDR has been reported to increase gene expression of enteric transporters including SLC30A10 (zinc and manganese transporter ZnT10) (Claro da Silva et al., 2016), ABCB1 (P-glycoprotein) (Tachibana et al., 2009), proton-coupled folate transporter (Eloranta et al., 2009), and apical sodium-dependent bile acid transporter (Chen et al., 2006).

2. Constitutive androstane receptor (CAR): CAR, the nuclear receptor responsible for CYP2B6 induction, is known to be expressed in human intestinal mucosa (and has been postulated to be involved in the regulation of P450 (Burk et al., 2004; Fritz et al., 2019) and efflux transporter MDR1 expression (Burk et al., 2005) in the human small intestines as observed for hepatocytes (Pascussi et al., 2000). A study comparing wild-type and *CAR* knockout mice has demonstrated a regulatory role of CAR on the expression of a variety of drug metabolism and transporter genes including Cyp2b10, Cyp3a11, Ugt1a1, Ugt2b34, Ugt2b36, and Mrp2-4 (Park et al., 2016). The exact role of CAR on enteric P450 gene expression in the human intestine is yet to be fully defined.
3. Pregnane X receptor (PXR): PXR, the key nuclear receptor for CYP3A and CYP27A1 induction, is highly expressed in human intestinal mucosa. Similar to PXR in the liver, enteric PXR expression is reported to be attenuated by inflammatory responses (Blokzijl et al., 2007; Shakhnovich et al., 2016; Deuring et al., 2019). Activation of CYP3A by rifampin, a model PXR ligand, has been observed in human intestinal biopsies (Burk et al., 2004).
4. Farnesoid X receptor (FXR): FXR is highly expressed in the small intestines with bile acids as endogenous agonists (Cariou and Staels, 2006). Enterohepatic recirculation and microbial metabolism of bile salts are reported to modulate FXR expression and subsequent events including the induction of an intestinal hormone, fibroblast growth factor 19, which activates hepatic fibroblast growth factor receptor 4 signaling to inhibit the hepatic bile acid synthesis by CYP7A (Chiang, 2009). In human hepatocytes, FXR activation has been reported to result in repression of CYP3A4 expression, presumably via enhanced expression of a repressor of multiple transcription factors, the small heterodimer partner (Zhang et al., 2015). FXR is believed to regulate the expression of the bile acid efflux transporters OSTα and OSTβ (Dawson et al., 2005; Ballatori et al., 2013).
5. Liver X receptor (LXR): In spite of having been named after the liver, LXRα is known to be expressed in numerous metabolically active tissues including liver and intestine, with oxidized cholesterols as endogenous ligands (Zhao and Dahlman-Wright, 2010). Intestinal LXR activation has been reported to be associated with decreased intestinal cholesterol absorption (Lo Sasso et al., 2010), mainly attributed to the upregulation of enteric efflux transporters responsible for reverse transport of cholesterol into the intestinal lumen (Plösch et al., 2002; Colin et al., 2008).
6. Aryl hydrocarbon receptor (AhR): AhR, originally discovered in the liver, is also found in extrahepatic tissues, including the intestine, with ligands including the environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (Csanaky et al., 2018) as well as dietary components such as tryptophan metabolites (Manzella et al., 2018). Besides the regulation of CYP1A expression (Do et al., 2012), AhR is now known to play an important role in immune modulation (Li et al., 2016; Ehrlich et al., 2017; Metidji et al., 2018) and the maintenance of barrier functions (Liu et al., 2018) in the intestine.
7. Peroxisome proliferator–activated receptors (PPARs): The three major PPARs are PPARα, PPARβ/δ, and PPARγ [nuclear receptor subfamily 1, group C, members 1, -2, and -3, respectively]. In humans, PPARα is present mainly in liver, heart, and kidney; PPARβ/δ is in all organs evaluated including intestine (Peters et al., 2019); and PPARγ in adipose tissue, large intestine, macrophages, monocytes, and Caco-2 cells (Dubuquoy et al., 2006; Couvigny et al., 2015; Fumery et al., 2017). Intestinal microbiota play key roles in intestinal inflammation via the production of PPAR agonists and antagonists, suggesting that PPAR may be involved in the pathophysiology of intestinal inflammatory diseases such as irritable bowel syndrome (Belmonte et al., 2012). Although activation of PPARa in hepatocytes is well established to lead to hepatocyte proliferation (Brocker et al., 2017) and CYP4α induction (Li and Chiang, 2006), the role of PPAR on the expression and activity of enteric drug metabolizing enzymes and transporters remains to be elucidated.

Enteric Drug Metabolizing Enzymes

It has been well established that the human small intestine possesses robust drug metabolizing enzyme activities. Reports on the gene expression, protein expression, and activity of the various drug metabolizing enzyme pathways in the human small intestine are reviewed here.

Clinical Findings

CYP3A. The robust enteric CYP3A activity has been demonstrated conclusively by the increased bioavailability of orally administered CYP3A substrate drugs upon coadministration of GFJ, a potent enteric CYP3A inhibitor in vivo. CYP3A substrate drugs with peak and area under the concentration time curve values increased upon oral co-administration with GFJ include sertraline (Ueda et al., 2009), itraconazole (Gubbins et al., 2008), triazola (Sugimoto et al., 2006), felodipine (Goosen et al., 2004), itraconazole (Gubbins et al., 2004), atorvastatin (Gubbins et al., 2004), and midazolam (Veronese et al., 2003).

UDP-Glucuronosyltransferase. Clinical pharmacokinetic studies with raloxifene show that UDP-glucuronosyltransferase (UGT) can play a significant role in the oral bioavailability of its substrates. Raloxifene has an extremely low (approximately 2%) oral bioavailability resulting from presystemic metabolism by UGT (Trdan et al., 2011), especially UGT1A1, UGT1A8, UGT1A10, and UGT1A28 (Mizuma, 2009; Trontelj et al., 2009).

In Vitro/Ex Vivo Findings

Activity. Early studies employing homogenates, postmitochondrial supernatents, and microsomes of human intestinal biopsy samples have been found to be active in phase 1 oxidation, including testosterone 6 beta-hydroxylase, (+)-bufuralol 1′-hydroxylase, carboxyesterse, 7-ethoxycoumarin *O*-deethylase, and 7-ethoxyresorufin *O*-deethylase (Hoensch et al., 1984; Kaminsky and Fasco, 1991; Prueksaritanont et al., 1996); piperidine *N*-dealkylation of fentanyl to norfentanyl (Labroo et al., 1997) as well as phase 2 conjugation including bilirubin-, 4-nitrophenol-, and 4-methylumbelliferone UDP-glucuronosyltransferase (Peters et al., 1989); and characterization of CYP3A4 activity (Paine et al., 1997). Reported drug metabolizing enzyme activities with intact cell systems including precision cut human intestinal slices, showing robust CYP2C9 and CYP3A4 but undetectable CYP2A6 activities (van de Kerkhof et al., 2006), and the metabolism of tegaserod, a selective 5-hydroxytryptamine receptor 4 partial agonist with promotile activity in the gastrointestinal tract, to the *N*-glucuronides (Vickers et al., 2001). In our laboratory, we have developed cryopreserved intact cell human enteric systems including cryopreserved purified human enterocytes (Ho et al., 2017), permeabilized cofactor-supplemented (MetMax) cryopreserved human enterocytes (Li et al., 2018b; Wong et al., 2018), and cryopreserved human intestinal mucosa (CHIM) (Li et al., 2018a; Zhang et al., 2020). These systems represent practical in vitro experimental systems for the definition of enteric drug metabolism activities. Using these systems,

robust activities have been detected in the human small intestines including both P450 and non-P450 drug metabolizing enzyme activities including CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2J2, CYP3A4, UGT, SULT, monoamine oxidase (MAO), FMO, CES, NAT1, and NAT2 activity (Tables 2 and 3) as well as confirming the lack of quantifiable CYP2A6 activities. The overall highest specific activity was observed for MAO, with CYP3A4 representing the P450 isoform with the highest activity. Using CHIM, UGT2B7 and UGT2B17 activities further defined using isoform selective substrates and inhibitors, with significant correlation with protein abundance based on proteomics (Zhang et al., 2020). The results with the P450 isoforms with the intact cell enteric models are similar to those reported for intestinal microsomes (Clermont et al., 2019).

Gene Expression. Gene expression has been applied extensively to identify metabolizing enzymes present in the human intestine, although it is generally accepted that gene expression results do not always correlate quantitatively with protein and activity (Hayashi et al., 2011). CYP2E1, CYP3A4, and CYP3A5 mRNA were found to express in gastric, duodenal, colonic, and rectal mucosa biopsies obtained during routine gastro-colonoscopy in 27 patients (Thörn et al., 2005). An independent finding with duodenum, jejunum (proximal and mid-jejunum), and ileum (proximal and mid-ileum) regions demonstrated expression of P450 mRNA ranked as follows: CYP3A4 > CYP2C9 > CYP2C19 > CYP2J2 > CYP4F2 (Clermont et al., 2019). Gene expression results with intestinal biopsies show that PXR, CYP3A4, and villin 1 expression was decreased in the inflamed small intestinal tissue in children with Crohn's disease (Shakhnovich et al., 2016), a result consistent with that observed in hepatocytes (El-Kadi et al., 1997; Assenat et al., 2004), suggesting that environment factors may have effects on the expression of drug metabolizing enzymes in the human small intestine via similar mechanisms as observed in the human liver.

Proteomics. Proteomics represent an important approach for the quantification of protein expression. A recent proteomics study on drug metabolizing enzymes in the human small intestinal mucosal tissues from 26 patients undergoing intestinal surgery confirms the presence of the various P450 (CYP2C9, CYP2C19, CYP2D6, CYP2J2, CYP3A4) and UGT (UGT1A1, UGT1A3, UGT1A6, UGT2B7, UGT2B15) isoforms, with CYP3A4 and with UGT1A1 as the most abundant isoforms, respectively (Couto et al., 2020).

Enteric Transporters

As an organ serving as a barrier between the environment and the systemic circulation specializing in nutrient absorption, the intestinal mucosal epithelium has evolved tight junctions to allow it to serve as a protective barrier as well as various transporters for the uptake and efflux of selective molecules that are relatively impermeable to the mucosal membranes. These transporters are now known to be involved in xenobiotic uptake and efflux of drug substances, environmental pollutants, and ingredients of botanical origin.

Clinical Findings

GFJ and Orange Juice Inhibition of Enteric Transporters. The significant role of enteric uptake and efflux transporters on drug absorption is illustrated by the clinically significant effects of GFJ and orange juice on the bioavailability of drugs that are uptake or efflux transporter substrates. Transporters identified as being inhibited by GFJ are listed below.

P-glycloprotein. P-gp is a protein located on apical membranes of enterocytes, serving to remove absorbed P-gp substrates from the cytoplasm back to the intestinal lumen. P-gp thereby can play an important role on the bioavailability of ingested xenobiotics. GFJ is now known to be a potent inhibitor of P-gp and CYP3A4 and has been found in clinical trials to enhance the systemic burden of orally administered P-gp substrates (which in general are also CYP3A substrates) including verapamil (Ho et al., 2000), diltiazem (Christensen et al., 2002), and cyclosporin (Brunner et al., 2000). In general, it is believed that both P-gp and CYP3A inhibition are key mechanisms for the GFJ effects.

OATP1A2. OATP1A2 is an uptake transporter located on the apical membranes of enterocytes. Clinical evidence for the involvement of OATP1A2 in drug uptake is provided by the observation that the plasma concentration of orally administered aliskiren, a substrate of OATP1A2, is significantly decreased upon coadministration with GFJ (Rebello et al., 2012) or orange juice (Tapaninen et al., 2011).

In Vitro/Ex Vivo Findings

Activity. Although enteric uptake and efflux transporters have been readily identified via gene expression and proteomics studies, at this writing, there are no in vitro experimental systems with the complete array of these transporters working in concert as in the human small intestine in vivo. Especially lacking is an in vitro system with both transporters and drug metabolizing enzymes allowing the evaluation of transport-drug metabolism interplay akin to the use of primary human hepatocytes for the evaluation of hepatic drug properties. Enteric P-gp efflux is routinely evaluated using Caco-2 transwell cultures, with known substrates and inhibitors showing the expected properties. Early studies with grapefruit juice in Caco-2 cells were instrumental in the development of the hypothesis that P-gp inhibition is a key mechanism for GFJ-drug interactions using various P-gp substrates including vinblastine (Takanaga et al., 1998), taninolol (de Castro et al., 2007), and digoxin (Xu et al., 2003), and the identification of 6′,7′-epoxybergamottin, 6′,7′-dihydroxybergamottin and naringenin as key P-gp inhibitors present in the grapefruit juice (de Castro et al., 2007). Enteric transporters responsible for drug uptake have also been identified using Caco-2 cells as exemplified by the identification of organic cation transporter 1 (OCT1; SLC22A1), plasma membrane monoamine transporter, serotonin reuptake transporter (SLC6A4), and choline high-affinity transporter (SLC5A7) for metformin uptake into the enterocytes (Han et al., 2015).

Gene Expression. Human small intestine has been reported to express mRNA for numerous transporter genes. Hilgendorf et al. (2007) reported that 26 of the 36 transporter genes evaluated were found in the jejunum, with the highly expressed being dipeptide uptake carrier PepT1 and the ABC efflux transporters multidrug-resistance protein (MRP) 2, MDR1, and BCRP and with undetectable bile salt export pump, sodium (Na+) taurocholate co-transporting polypeptide, OCT2, and OAT1. Drozdzik et al. (2019) compared human liver and intestinal tissues and reported the expression of P-gp, MRP2, MRP3, MRP4, BCRP, OATP2B1, OCT1, apical sodium-bile acid transporter, MCT1, and peptide transporter (PEPT1) in the intestine, with hepatic gene expression found to be substantially higher than enteric gene expression for MRP2, OCT1, and OATP2B1. Kim et al. (2007) evaluated transporter gene expression in human duodenum with the highly expressed genes being peptide transporter HPT1; amino acid transporters LAT3, 4F2HC, and PROT; nucleoside transporter CNT2; organic cation transporter OCTN2; organic anion transporters NADC1, NBC1, and SBC2; glucose transporters SGLT1 and GLUT5; multidrug resistance-associated protein RHO12; fatty acid transporters FABP1 and FABP2; and phosphate carrier PHC.

Proteomics. The protein abundance of enteric transporters can be quantified by proteomic approaches. Besides the evaluation of gene expression as described above, Kim et al. (2007) also evaluated liver and intestinal expression of transporters based on protein abundance detected by liquid chromatography–tandem mass spectrometry and found similar relative abundance for protein and mRNA. A recent report by Couto et al. (2020) quantified protein abundance of various

TABLE 2

Drug metabolizing enzyme activities (picomoles per minute per milligram protein) in CHIM

Means and S.E.s (S.E.M.) of CHIM from four donors are shown. The results are derived from those previously published (Li et al., 2018a).

Drug metabolizing enzyme	Substrate	Substrate conc. (μM)	Marker metabolite	Mean	S.E.M.
CYP1A1	7-Ethoxyresozufin	20	Resozufin	0.26	0.13
CYP1A2	Phenacetin	100	Acetaminophen	3.34	0.89
CYP2A6	Coumarin	50	7-HC, 7-HC-sulfate, 7-HC-glucuronide	NA	NA
CYP2B6	Buproprion	500	Hydroxybuproprion	0.69	0.11
CYP2C8	Paclitaxel (taxol)	20	6α-Hydroxypaclitaxel	0.12	0.02
CYP2C9	Diclofenac	25	4-OH diclofenac	0.39	0.05
CYP2C19	*S*-mephenytoin	250	4-OH *S*-mephenytoin	0.86	0.26
CYP2D6	Dextromethorphan	15	Dextrophan	0.04	0.02
CYP2E1	Chlorzoxazone	250	6-OH chlorzoxazone	0.04	0.01
CYP2J2	Astemizole	50	*O*-demethyl astemizole	2.27	0.31
CYP3A4-1	Midazolam	20	1-Hydroxymidazolam	1.83	0.65
CYP3A4-2	Testosterone	200	6β-Hydroxytestosterone	24.82	5.47
ECOD	7-Ethoxycoumarin	100	7-HC, 7-HC-sulfate, 7-HC-glucuronide	0.81	0.24
UGT	7-Hydroxycoumarin	100	7-Hydroxycoumarin glucuronide	0.71	0.26
SULT	7-Hydroxycoumarin	100	7-Hydroxycoumarin sulfate	1.85	0.44
GST	Acetaminophen	10 mM	Acetaminophen glutathione	0.26	0.09
UGT	Acetaminophen	10 mM	Acetaminophen glucuronide	1.04	0.51
SULT	Acetaminophen	10 mM	Acetaminophen sulfate	4.52	0.84
FMO	Benzydamine HCl	250	Benzydamine-*N*-oxide	8.11	0.99
MAO	Kynuramine HBr	160	4-Hydroxyquinoline	317.09	80.12
AO	Cabazeran	20	4-Hydroxycabazeran	0.02	0.00
NAT1	4-Aminobenzoic acid	200	*N*-acetyl-p-aminobenzoic acid	2.17	0.58
NAT2	Sulfamethazine	100	*N*-acetyl-sulfamethazine	1.46	0.16
CES2	Irinotecan	50	SN38	1.21	0.13

ECOD, 7-ethoxycoumarin *O*-deethylase; NA, no activity.

transporters in human jejunum and ileum, showing similar expression for the two regions of the small intestine, with MRP2, BCRP, OST-α, OST−β, OATP1A1, and OATP2B1 being the most abundant and poor correlation with mRNA expression except for P-gp and OST-α.

In Vitro Human Cell–Based Enteric Systems

As reviewed above, the intestinal mucosal epithelium is responsible for absorption and metabolism of orally administered substances. An ideal in vitro enteric experimental system is one that can model the various events that occur in vivo including absorption, metabolism, and efflux (Fig. 1) via the expression of in vivo levels of the key components of the intestinal mucosal epithelium, including nuclear receptors, drug metabolizing enzymes, and uptake and efflux transporters. For all the key components to be functional and interactive as in vivo, intact cells are required rather than cell-free systems such as cell homogenates, postmitochondrial supernatants, and microsomes, as exemplified by intact human hepatocytes versus cell-free hepatic systems (Li, 2005; Hewitt et al., 2007). The currently available human cell–based enteric systems are reviewed below.

TABLE 3

A comparison of cryopreserved human enterocytes, MetMax cryopreserved human enterocytes, and CHIM in the major drug metabolizing enzyme pathways

The specific activities for CHE, MMHE, and CHIM were extracted from previously published data (Ho et al., 2017; Li et al., 2018a,b), with the data for CHE and MMHE converted from picomoles per minute per million cells to picomoles per minute per milligram. The numbers of donors for the calculation of mean activities were 25 (CHE), 10 (MMHE), and 3 (CHIM). CHE and MMHE were prepared from the entire small intestines. CHIM were prepared from the duodenum (D), jejunum (J), and ileum (I).

DME	Pathway	Mean specific activity (pmol/min per milligram protein)				
		CHE	MMHE	CHIM(D)	CHIM(J)	CHIM(I)
CYP2C9	Diclofenac 4-hydroxylation	0.6	2.9	2.1	5.3	5.2
CYP2C19	*S*-mephenytoin 4-hydroxylation	0.2	1.7	5.9	2.0	1.1
CYP2J2	Astemizole *O*-demethylation	0.4	2.8	2.5	2.7	3.5
CYP3A	Midazolam 1′-hydroxylation	0.5	2.1	4.7	8.2	5.0
CYP3A	Testosterone 6β-hydroxylation	10.0	73.5	91.3	86.5	42.4
UGT	7-Hydroxycoumarin glucuronidation	7.2	137.5	7.2	4.0	11.3
SULT	7-Hydroxycoumarin sulfation	2.9	6.5	3.2	1.2	0.4

CHE, cryopreserved human enterocyte; DME, Drug metabolizing enzyme; MMHE, MetMax cryopreserved human enterocyte.

Cell Lines

Caco-2

Findings with Caco-2 cells are reviewed here as research with this cell line contributes extensively to our understanding of intestinal drug absorption. Caco-2 cells were originally cloned from a human colorectal adenocarcinoma culture and later found to differentiate to express enterocyte properties upon prolonged (3–4 weeks) culturing as confluent monolayer cells (Hidalgo et al., 1989). For the evaluation of intestinal permeability, Caco-2 cells are cultured in transwell consisting of an upper (apical) well with a semipermeable membrane modeling the intestinal lumen and a lower (basolateral) well modeling the enteric blood compartment connecting to the systemic circulation via the portal circulatory system. Upon prolonged culturing (approximately 14–21 days), the Caco-2 cells form confluent, polarized monolayer cultures with tight cell-cell junctions akin to those found in the intestinal mucosal epithelium. The drug to be evaluated is added to the apical chamber and its intestinal permeability is determined by quantifying its appearance in the basolateral chamber. Caco-2 cells express both uptake and efflux transporters, especially the key enteric efflux transporter P-gp (MDR1) (Seithel et al., 2006). The strengths of the Caco-2 transwell system include the well established experimental protocol with an extensive database for myriads of drug substances showing relatively good correlation with clinical oral availability. An important application of

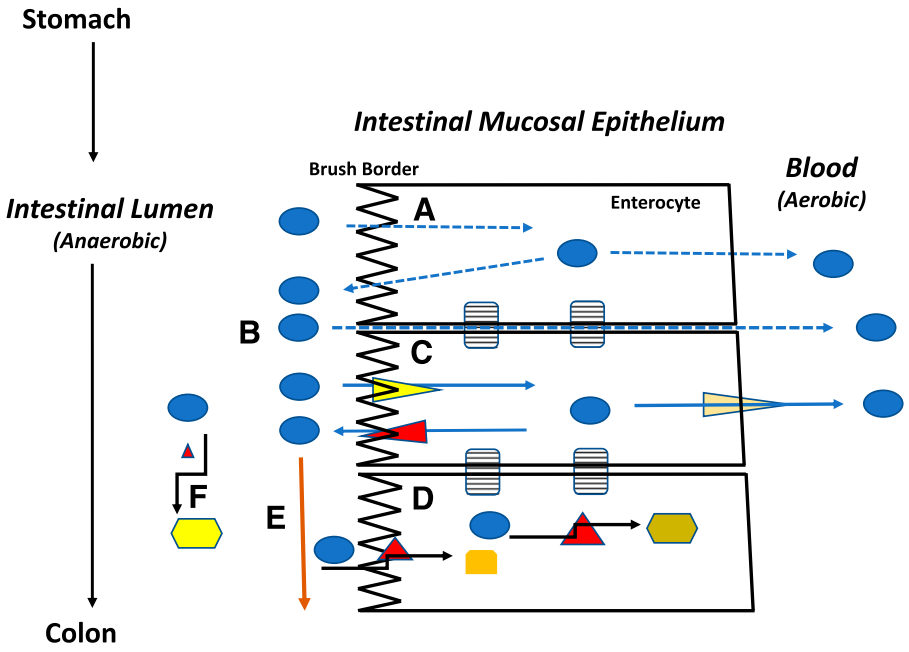

Fig. 1. Schematic representation of the enteric compartments and key events that occur upon oral administration of xenobiotics (blue circles) as a blueprint for a "complete" in vitro experimental system for the evaluation of enteric drug metabolism. The three major compartments are 1) the intestinal lumen with the resident microbiome in an anaerobic atmosphere, 2) the mucosal epithelium, and 3) the systemic circulation (blood). Not represented are the mucus layer, nonenterocytes in the mucosa, and blood vessels. Upon ingestion, the xenobiotics are subjected to absorption into the intestinal mucosal epithelium via concentration-dependent diffusion across the enteric cells (transcellular diffusion; A) or between enteric cells across cell-cell junctions (paracellular diffusion; B). Xenobiotics that are not freely permeable to the plasma membranes can enter the enterocytes via transporter-mediated uptake (C) and, upon entering the enterocytes, may exit back to the lumen or into the enteric circulation via transporter-mediated transport. An orally administered xenobiotic is subjected to metabolism by the drug metabolizing enzymes in the brush border and enterocytes (D) upon entering the mucosal epithelium or by intestinal microflora in the intestinal lumen, with the subsequent metabolite subjected to the various uptake and efflux pathways followed by either entering the enteric blood circulation or excretion via the colon (E). The schematic illustrates the various processes required to be modeled by an in vitro enteric experimental system as well as the challenges of developing a single system to model all the key processes.

the Caco-2 in vitro permeability assays is the Biopharmaceutics Classification System and Biopharmaceutics Drug Disposition Classification System, in which drugs are classified based on solubility, permeability, and metabolic clearance for drug development and regulation (Benet, 2013). Caco-2 cells are reported to express low levels of the key enteric drug metabolizing enzyme, CYP3A4 (Raeissi et al., 1999; Fan et al., 2009; Sergent et al., 2009), which can be further enhanced by transfection with CYP3A4 (Cummins et al., 2004) or nuclear receptors (Korjamo et al., 2006), subcloning (Raeissi et al., 1999), or the addition of CYP3A4 inducers in the culture medium (Aiba et al., 2005). Caco-2 cells are considered an important in vitro experimental system for the evaluation of oral drug permeability and P-gp–mediated efflux, but not for enteric drug metabolism due to low endogenous drug metabolizing enzyme activities.

Non–Caco-2 Cell Lines

Besides Caco-2 cells, cell lines used for the evaluation of intestinal physiology and functions include those derived from human colon carcinoma (HT29, HRT-18, HCT-8R, SW-480, and CO-115) (Zweibaum et al., 1983). As these cell lines provide information similar to that obtained Caco-2 cells, they are not included in this review.

Stem Cell–Derived Models

Crypt Cell Enteric Systems. The surface area of the small intestine is greatly increased due to the presence of villi, finger-like projections into the intestinal lumen composed of enterocytes, and further increased by the presence of microvilli, microscopic projections on the cell surface of the enterocytes. The enterocytes of each of the villus originate from the stem cells situated at the crypts (crypts of Lieberkuhn), the pits between villi. Crypt cell–derived enterocytes continue to migrate and mature up the villus and eventually enter into apoptosis and slough off into the intestinal lumen at the tip. The journey from the crypt to detachment from the tip takes approximately 4 to 5 days (Vachon et al., 2000; van der Flier and Clevers, 2009). Crypt cells from the human small intestine have been successfully cultured as primary monolayer cells (Browning and Trier, 1969; Panja, 2000; Benoit et al., 2010; Beaulieu and Ménard, 2012) as well as three-dimensional organoids (enteroids) as an in vitro model for the evaluation of intestinal physiology and diseases including the evaluation of major histocompatibility complex class II regulation (Wang et al., 2018; Wosen et al., 2019), interaction of the enterocytes with pathogenic microbiota (In et al., 2019; Stewart et al., 2020), modeling infectious diarrheal diseases (Kovbasnjuk et al., 2013; Foulke-Abel et al., 2014, 2016), elucidation of pathogenesis of intestinal diseases such as inflammatory bowel disease (Rees et al., 2019) and necrotizing enterocolitis (Senger et al., 2018; Ares et al., 2019), and the elucidation of the cell and molecular pathways controlling stem cell maturation into enterocytes (Das et al., 2015; Mahe et al., 2015; Schilderink et al., 2016). As of this writing, there are no reports defining the expression and activity of drug metabolizing enzymes in human enteroids.

Induced Pluripotent Stem Cell Enteric Systems. Differentiation of induced pluripotent stem cells (iPSCs) into organ-specific cell types represents a significant scientific achievement with important medical and biomedical applications including cell therapy and in vitro evaluation of organ-specific biology and drug properties. Technological advancement continues to be made to improve the efficiency of the differentiation of iPSCs to various differentiated cell types including three-dimensional beating cardiomyocytes (Sasano et al., 2020), hepatocytes (Takayama et al., 2018), neurons (Cheng et al., 2017), and three-dimensional kidney organoids (Takasato et al., 2016). Successful differentiation of iPSCs into enterocytes has also been reported (Iwao et al., 2014, 2015; Negoro et al., 2016; Blutt et al., 2017; Nadkarni et al., 2017; Uchida et al., 2017; Kondo et al., 2018; Macedo et al., 2018; Lees et al., 2019; Kondo et al., 2020), including three-dimensional enterocyte organoids (Onozato et al., 2018). The efficiency of enterocyte differentiation from human iPSCs was significantly improved by advancements in culture medium formulation and differentiation protocol (Ozawa et al., 2015). Enterocytes derived from iPSCs express key enterocyte

markers including PEPT1, MDR1, MRP3, OATP2B1, EAAC1, TAUT, CYP3A4. CYP2E1, and CES2 (Ogaki et al., 2015). The iPSC enterocytes represent a significant scientific achievement with promising applications toward the evaluation of drug-induced mucosal damage and intestinal permeability (Ozawa et al., 2015; Kondo et al., 2018). A major challenge with the iPSC enterocytes is that the drug metabolizing enzyme activities are not fully characterized. A report on CYP3A activity, for instance, shows extremely low activities using luciferin isopropyl acetal as substrate, with luciferin formation detected only after a prolonged incubation of 24 hours (Iwao et al., 2015). At the time of this writing, iPSC enterocytes are not yet readily applicable for the evaluation of the metabolic fate of orally administered drugs.

Primary Human Enteric Models

Intestinal Slices. Tissue slices, pioneered by Klaus Brendel, represent an important approach for in vitro evaluation of organ-specific drug properties, including drug metabolism, pharmacology, and toxicity (Smith et al., 1985; Brendel et al., 1987). Intestinal slices have been successfully applied in the evaluation of organ and species differences in the metabolism of various drugs including cyclosporin A and its analog (Vickers et al., 1992, 1995), lidocaine (De Kanter et al., 2002), and quinidine (Li et al., 2017). Appropriately prepared human intestinal slices without extensive tissue damage should retain all enteric drug metabolizing enzyme activities and therefore be appropriate for use in the evaluation of enteric drug metabolism. A practical drawback is that fresh human intestine is required for the application of human intestinal slices in experimentation.

Primary Enterocytes/Intestinal Mucosa Isolates. Successful cryopreservation of human hepatocytes to retain viability and various key hepatic functions including transporter-mediated uptake and efflux, drug metabolism, and response to enzyme and transporter inhibitors and inducers is a major reason for this experimental system to be considered the "gold standard" in vitro experimental system for the evaluation of human hepatic drug metabolism (Li et al., 1997, 1999; Hewitt et al., 2007; Li, 2007, 2008, 2010, 2014). We thereby apply this approach toward the development of in vitro enteric models for drug metabolism studies.

Below is a review of the three experimental systems developed recently in our laboratory, namely, cryopreserved human enterocytes, permeabilized cofactor-supplemented (MetMax) human enterocytes, and cryopreserved human intestinal mucosa. The systems were developed using human small intestines procured but not used for transplantation (provided to our laboratory by the International Institute for the Advancement of Medicine, Edison, NJ). The schematic illustrating the preparation of three enteric systems from the human intestine is shown in Figure 2.

Cryopreserved human enterocytes. Cryopreserved human enterocytes (Ho et al., 2017) were isolated via collagenase digestion of the intestinal mucosa. The enterocytes were purified by density gradient centrifugation and the purity of the cells have been validated via morphology (showing homogenous cell size), enzyme activities (alkaline phosphatase; P450), and gene expression (gene markers including sucrose isomaltase; maltase glucoamylase). Upon recovery from cryopreservation, the enterocytes retain over 50% viability and express robust CYP2C9, CYP2C19, CYP2J2, CYP3A4, CYP2J2, UGT, and SULT activities (Table 3). The cryopreserved enterocytes, as purified cells, have been applied in a proteomics study (Zhang et al., 2018), demonstrating correlation between protein abundance and activity of CYP2B17 for enterocytes from 16 human donors. The robust drug metabolizing enzyme activities of the cryopreserved human enterocytes allow them to be used to evaluate the metabolic fate of orally administered drugs. Challenges to cryopreserved human enterocytes include the following: 1) Low yield: Due to the need to enzymatically digest the intestinal mucosa to single cells and to purify the enterocytes (via density gradient) from the nonenterocytes, the yield per human intestine is no more than 500 million enterocytes from the entire human small intestine including duodenum, jejunum, and ileum. 2) Limited culture duration: As of this writing, we are only able to use the enterocytes for short-term (up to 4 hours) incubation. The enterocytes do not attach and cannot be maintained as long-term cultures. 3) Relatively low drug metabolizing enzyme activities: Although there are the expected donor-to-donor differences, the cryopreserved enterocytes in general have lower drug metabolizing enzyme activities than the MetMax enterocytes and the cryopreserved intestinal mucosa described below.

Permeabilized cofactor-supplemented (MetMax) cryopreserved human enterocytes. MetMax cryopreserved human enterocytes (Li et al., 2018b) were prepared using a proprietary technology previously developed for MetMax cryopreserved human hepatocytes (Li et al., 2018c). MetMax cryopreserved human enterocytes are prepared via the permeabilization of the plasma membrane of intact cryopreserved human enterocytes. The major purpose of the modification of the intact cell system is to enhance the ease of application: MetMax hepatocytes and enterocytes can be stored at −80°C instead of with liquid nitrogen and can be used immediately upon thawing without the relatively cumbersome procedures of centrifugation and microscopic cell counting and viability determination as required for conventional cryopreserved cells. One advantage of MetMax metabolic systems is that the cells are supplemented with phase I oxidation and phase II conjugation cofactors for use in the evaluation of overall drug metabolism; thereby drug metabolism pathways can be selected via the use of specific cofactor mixtures. Another significant advantage over the intact cryopreserved enterocytes is that the metabolic capacity of MetMax cells is not affected by the cytotoxicity of the test substance—a major complication with the application of intact cell systems to evaluate drug metabolism at cytotoxic drug concentrations. The MetMax cryopreserved human enterocytes possess the same metabolizing enzymes as the intact cryopreserved human enterocytes but with a higher activity (Table 3), presumably due to the presence of optimal cofactor concentrations. The MetMax human enterocytes have recently been applied toward the evaluation of intrinsic enteric metabolic clearance of various model compounds and compared with intrinsic hepatic clearance using cryopreserved human hepatocytes (Wong et al., 2018), showing that enteric metabolism can be higher or lower than hepatic metabolism for various drugs. Enteric clearance was found to be lower than hepatic clearance for the CYP3A4/5 substrates midazolam, amprenavir, and loperamide and for procaine, a CES2 substrate. Enteric clearance was found to be substantially higher than hepatic clearance for raloxifene, a UGT activity substrate. Salbutamol, a SULT1A3 substrate, was metabolized to the sulfate conjugate at a higher abundance than hepatocytes. The MetMax human enterocytes represent a practical in vitro enteric system for the evaluation of metabolic fate and enteric drug-drug interactions of orally administered drugs and should be applicable toward the evaluation of enteric metabolic fate and drug interaction potential of botanical-based herbal medicine and health supplements. A major challenge to the use of MetMax enterocytes is that, due to the permeabilized plasma membrane, the experimental system cannot be used for the evaluation of transporter-mediated uptake and efflux.

Cryopreserved human intestinal mucosa. CHIM (Li et al., 2018a) are prepared from the entire human mucosal epithelium without further cell separation and purification and thereby contain all cell types in the intestinal mucosa, representing a relatively complete experimental model for the intestinal mucosa. The human intestinal mucosal epithelium is detached from the intestinal lumen via collagenase

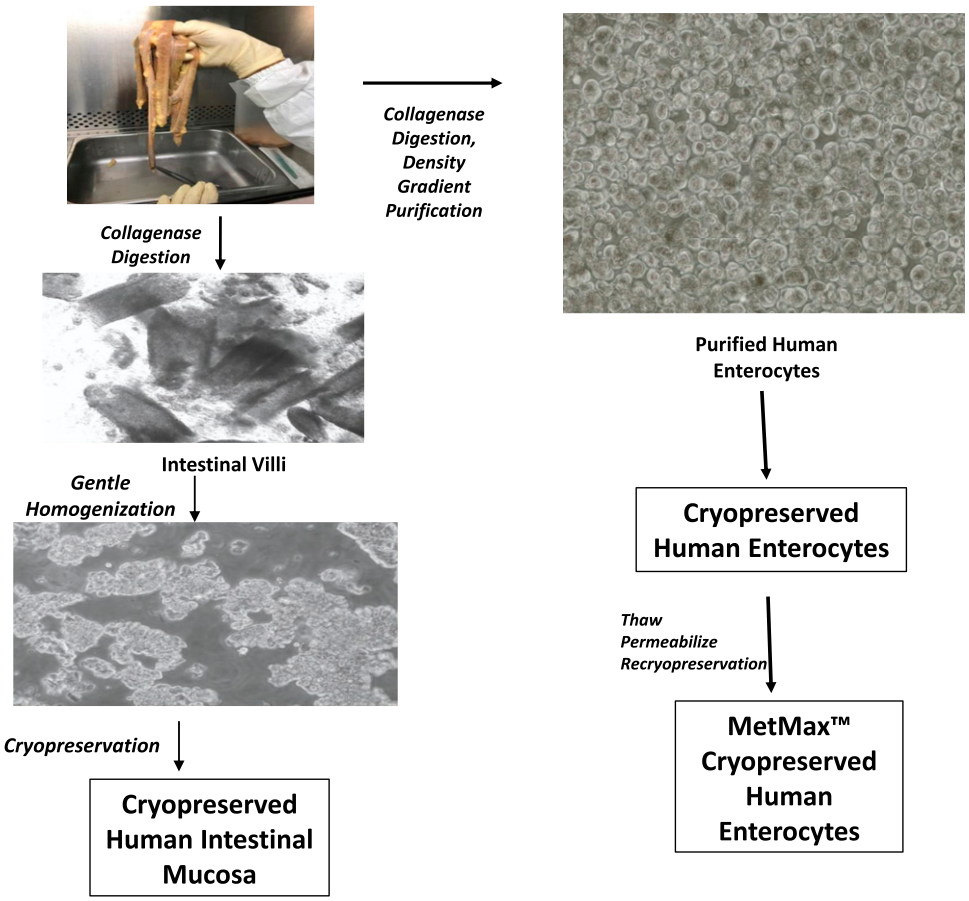

Fig. 2. Schematics for the isolation and cryopreservation of human enterocytes, MetMax human enterocytes, and cryopreserved human intestinal mucosa. The schematics are modified from those previously published (Ho et al., 2017; Li et al., 2018a,b). Collagen digestion of the human intestinal lumen releases the intestinal mucosa, which are then further digested with collagenase to yield single cell suspensions of enterocytes, which are purified by density gradient centrifugation, followed by cryopreservation as cryopreserved human enterocytes. For the preparation of MetMax human enterocytes, the cryopreserved enterocytes are recovered from cryopreservation, permeabilized, and recryopreserved. Cryopreserved human intestinal mucosa are prepared by gently homogenizing the human intestinal mucosal epithelium followed by cryopreservation.

digestion. The mucosa preparation is then homogenized with a loose-fitting Dounce homogenizer to small, multicellular fragments that can be readily delivered with a micropipette. CHIM have been shown to retain robust drug metabolizing enzyme activities including those for CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A4, UGT, SULT, FMO, AO, CES2, NAT1, and NAT2 (Table 2). In our laboratory, we have applied CHIM in the comparison of the cytotoxic potential of the anti-inflammatory drugs naproxen and acetaminophen, with results showing a higher cytotoxic potential of naproxen than acetaminophen (Li et al., 2018a), a result similar to the comparative gastrointestinal toxicity of the two nonsteroidal anti-inflammatory drugs in human in vivo, suggesting that CHIM may be applied toward the evaluation of the enterotoxic potential of orally administered drugs, especially for nonsteroidal anti-inflammatory drugs (Willett et al., 1994; Dobrilla et al., 1997; Dajani, 1998; Bjarnason and Thjodleifsson, 1999; Tenenbaum, 1999; Goldstein, 2004; Goldstein and Cryer, 2015). We have demonstrated CYP3A4 induction by rifampin and vitamin D3 (Li et al., 2018a) and CYP3A inhibition by grapefruit juice and several commercially available herbal supplements (Loretz et al (in press)). CHIM therefore represents an appropriate in vitro enteric experimental system for the evaluation of enteric drug properties including drug metabolism, drug-drug interactions, toxicity, and pharmacology. A major challenge with CHIM is that enterocyte-specific events cannot be readily identified as this experimental model consists of all cell types present in the intestinal mucosa.

A comparison of the procedures involved in the preparation of cryopreserved enterocytes, MetMax cryopreserved enterocytes, and CHIM is presented in Figure 2, and the procedures involved in the application of these systems to evaluate drug metabolism and drug-drug interactions are presented in Figure 3. A comparison of drug metabolizing enzyme activities of the three systems is shown in Table 3. The advantages and challenges for each category of the in vitro experimental systems reviewed are shown in Table 4.

Discussion

The human small intestine serves as a gateway for orally administered substances to enter the systemic circulation. Clinical findings, especially those concerning the effect of grapefruit juice on oral bioavailability of drugs that are substrates of CYP3A and the efflux transporter P-gp, demonstrate clearly that enteric drug transporters and drug metabolizing enzymes play an important role in bioavailability in additional to permeability. Investigations on human enteric drug properties can be greatly enhanced by human-based in vitro experimental systems modeling key determinants of bioavailability, namely, concentration diffusion via transcellular uptake across the enterocytes or paracellular uptake through the cell-cell junctions, transporter-mediated uptake and efflux, and enteric drug metabolism by the microflora in the intestinal lumen and enterocytes (Fig. 1).

Caco-2 transwell culture is well recognized for its application in the definition of intestinal permeability, including the evaluation of transporter-mediated uptake and efflux. Caco-2 cells also represent an important in vitro experimental system for the evaluation of transporter-mediated drug-drug and food-drug interactions, especially those involving P-gp inhibition. Successful transfection of Caco-2 with individual P450 isoforms, especially CYP3A4, allow this experimental model to be used to define the transporter-drug metabolism interplay, especially the interplay between CYP3A4 and P-gp. However, due to the

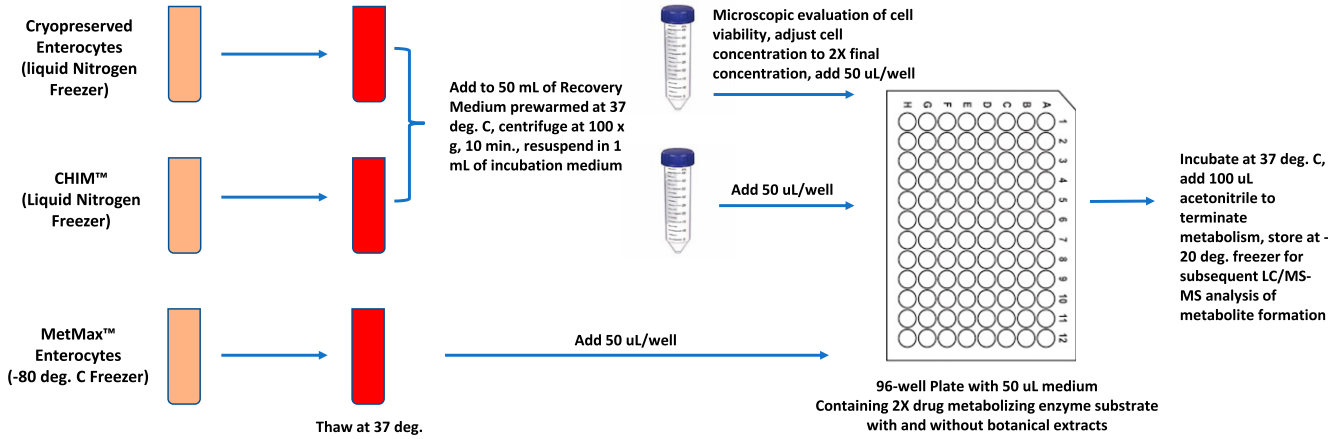

Fig. 3. Experimental procedures for the application of cryopreserved human enterocytes, CHIM, and MetMax cryopreserved human hepatocytes in the evaluation of the effects of botanical extracts on enteric metabolism. Cryopreserved human enterocytes and CHIM are stored in liquid nitrogen, whereas MetMax is stored in a −80°C freezer. Upon thawing, cryopreserved human enterocytes and CHIM are added to a recovery medium (50 ml) and centrifuged at 100*g* for 10 minutes, with the pellet resuspended in an incubation medium. Cryopreserved enterocytes are evaluated microscopically for viability (trypan blue exclusion) and cell concentration followed by adjustment of the cell concentration to 2× the final incubation (e.g., 2 million cells/ml for a final concentration of 1 million cells/ml), whereas CHIM is cryopreserved at 2 mg protein/ml and is resuspended in 1 ml of incubation to constitute a final protein concentration of 2 mg/ml, 2× the final concentration of 1 mg/ml. MetMax requires the least manipulation. It is cryopreserved at a cell density of 2 million cells/ml so can be added directly as a 2× cell suspension to the reaction plate. In this example, the wells of a 96-well plate are first loaded with medium containing 2× drug metabolizing enzyme substrate and botanical extract for the evaluation of potential P450 inhibition potential (e.g., using midazolam and enzyme substrate). After addition of the enteric suspension, the reaction plate is returned to a cell culture incubator. At the end of the incubation period, acetonitrile containing internal standard is added to each well followed by processing for liquid chromatography–tandem mass spectrometry (LC-MS/MS) quantification of metabolite formation.

presence of incomplete drug metabolizing enzyme activities, Caco-2 cells are not appropriate for the definition of enteric drug metabolism and pharmacokinetic drug-drug interactions per se.

Stem cell–derived human in vitro models represent promising approaches for the development of organ-specific cultures. In general, most organ-specific markers can be expressed with the exception of drug metabolizing enzymes, especially P450 isoforms. This is the major challenge in the application of iPSC-derived human hepatocytes and human enterocytes in the evaluation of events related to drug metabolism, such as pharmacokinetic drug interactions, and metabolic activation of prodrugs and protoxicants. Overcoming this challenge will greatly enhance the utility of the iPSC-derived cells for drug metabolism studies.

An ideal in vitro experimental model for the evaluation of human drug metabolism should have drug metabolizing enzyme pathways with activities similar to those in vivo. As of this writing, intestinal slices and primary enterocyte isolates—cryopreserved human enterocytes, MetMax cryopreserved human enterocytes, and cryopreserved human intestinal mucosa—represent appropriate experimental models for this application. Drug properties that can be defined with these experimental systems include enteric clearance, enteric metabolite profiling, enterotoxicity, and potential enteric drug-drug interactions. These models can be readily applied toward the evaluation of natural products, with an immediate important application in the definition of herb-drug interactions. The cryopreserved enteric systems have the advantage of the convenience of long-term storage in a laboratory and can be recovered and used as needed for experimentation. Tissue slices require preparation on the day of use, which may be challenging for human small intestines due to limited availability.

A relevant application of human enteric systems is the evaluation of drug interaction potential of herbal products. Herbal products are primarily delivered orally. Each herbal product has myriad components. Enteric herbal-drug interactions may occur resulting from the activity (e.g., CYP3A induction and inhibiton) of some of the components, either individually or working synergistically. As observed for grapefruit juice, these components may not enter into the portal circulation with plasma concentrations that would elicit effects on hepatic drug metabolizing enzymes. Due to their active drug metabolizing enzyme activities, human intestinal slices, cryopreserved human enterocytes, MetMax cryopreserved human enterocytes, and cryopreserved human intestinal mucosa are promising experimental systems for the evaluation of natural product-drug interactions.

As illustrated in Figure 1, an ideal in vitro enteric experimental model would be one with the various intestinal compartments, with enterocytes cultured as polarized cells with the apical side facing the anaerobic intestinal lumen containing intestinal microbiome, and the basal side adjacent to the blood, with the enterocytes exhibiting barrier functions, uptake and efflux transporters, and drug metabolizing enzymes. For enteric models derived from cell lines, crypt cells, and stem cells, emphasis should be placed toward to the development of models with adequate expression of enteric drug metabolizing enzymes and transporters. In our laboratory, efforts will be focused on the development of experimental approaches to evaluate uptake and efflux transport to further extend the application of cryopreserved human enterocytes and cryopreserved human intestinal mucosa in the assessment of human enteric drug properties. An ultimate goal of our laboratory is to develop approaches for the culturing of primary human enterocytes to express the key features of the intestinal mucosal epithelium in vivo, namely, tight cell-cell junctions, uptake transporters, drug metabolizing enzymes, and efflux transporters. Further improvements will be the inclusion of intestinal microbiome and the engineering of a culture vessel with the lumen compartment maintained in an anaerobic atmosphere with an aerobic atmosphere for the cell and blood compartments. Before the achievement of this ideal in vitro enteric system, one can apply each in vitro system to obtain information for each key event, and integrate the various data sets mathematically using various available physiologically based pharmacokinetic and in vitro–in vivo correlation approaches to provide an accurate assessment of the in vivo enteric properties of an orally administered xenobiotic.

TABLE 4

A comparison of the various in vitro experimental systems in their applications, strengths, and limitations for the evaluation of enteric drug properties

Model classification	Experimental systems	Applications							Strengths	Limitations
		Permeability	Uptake transport	Efflux transport	Drug metabolism	Metabolic DDI	Transporter DDI	Enterotoxicity		
Cell lines	Caco-2	Yes	Yes	Yes (P-gp)	No	No	Yes	Limited due to incomplete drug metabolism enzyme pathways	Extensive database; applied extensively in BCS drug characterization; expression of uptake and efflux transporters	Requires prolonged culturing (14 days) for establishment of barrier characteristics Lacks drug metabolizing enzymes
Crypt cell/ iPSC–derived models	Enteroids	Possible	Possible	Yes	No	No	Possible	Limited due to incomplete drug metabolism enzyme pathways	Human enteric cells appropriate for the elucidation of enteric differentiation	Lacks drug metabolizing enzyme activities
	Monolayer cultures	Possible	Possible	Yes	No	No	Possible	Limited due to incomplete drug metabolism enzyme pathways		
Primary cell/ organ models	Intestinal slices	Possible	Possible	Possible	Yes	Yes	Possible	Yes	Complete enteric drug metabolizing enzyme activities	Requires slice preparation on day of experimentation
	Cryopreserved enterocytes	Possible	Possible	Possible	Yes	Yes	Possible	Yes	Complete enteric drug metabolizing enzyme activities	Relatively low drug metabolizing enzyme activities compared with MetMax enterocytes and CHIM
	MetMax enterocytes	Possible	No	No	Yes	Yes	No	Yes	Complete enteric drug metabolizing enzyme activities; simple use procedures	Cannot be used for transport studies due to permeabilized plasma membranes (but can be used in conjuction with intact enterocytes to delineate role of permeability on experimental endpoints)
	Cryopreserved intestinal mucosa	Possible	Possible	Possible	Yes	Yes	Possible	Yes	Complete enteric drug metabolizing enzyme activities; complete mucosa allowing experimentation with all mucosal cell types	Approaches for uptake and efflux transport, though theoretically feasible, have not yet been developed

DDI, drug-drug interactionBCS, biopharmaceutics **classification** system

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