Minireview

Profiling of Drug-Metabolizing Enzymes and Transporters in Human Tissue Biopsy Samples; A Review of the Literature

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Abbreviations: ABC, ATP-binding cassette; A33, antigen 33; AUC, area under the plasma concentration versus time curve; BCRP, breast cancer resistance protein; CYP, cytochrome P450; LC/MS, tandem liquid chromatography-mass spectrometry; miR, microRNA; mRNA, messenger ribonucleic acid; MRP, multidrug resistance-associated protein; NASH, non-alcoholic steatohepatitis; NCE, new chemical entity; OATP, organic anion transporting polypeptide; PBPK, physiologically-based pharmacokinetics; Pgp, P-glycoprotein; PK, pharmacokinetics; PK-ADME, pharmacokinetics-absorption, distribution, metabolism, and excretion; SLC, solute carrier; UGT, UDP-glucuronosyltransferase.

Abstract

Within the drug pharmacokinetics-absorption, distribution, metabolism and excretion (PK-ADME) research community, investigators regularly generate in vitro data sets using appropriately vendor-sourced and processed human tissue. Such data enable drug screening, the generation of kinetic parameters, extrapolation of in vitro to in vivo, as well as the modeling and simulation of drug pharmacokinetics (PK). Although there are large numbers of manuscripts describing studies with deceased organ donor tissue, relatively few investigators have published studies utilizing living donor tissue biopsy samples. After a review of the available literature, it was possible to find publications describing the use of tissue biopsy samples to determine enzyme inhibition ex vivo, the study of genotype-phenotype associations, the evaluation of tissue expression profiling following an inducer, and assessment of correlations between tissue expression profiles and in vivo-derived trait measures (e.g., biomarker plasma levels and probe drug PK). Some reports described multiple single tissue biopsies, while others described single multiple organ biopsies. It is concluded that biopsy-derived data can support modeling exercises (as input data and when validating models) and enable the assessment of organ-specific changes in enzyme and transporter profiles resulting from drug interactions, disease (e.g., metabolic disease, fibrosis, inflammation, cancer, infection), age, pregnancy, organ impairment, and genotype. With the emergence of multi-organ axes (e.g., microbiome-gut-liver-kidney) and interest in remote sensing (inter-organ communication), it is envisioned that there will be increased demand for single and multi-organ tissue biopsy data to support hypothesis testing and PK-ADME model building.

Significance Statement

Based on a review of the literature, it is apparent that profiling of human tissue biopsy samples is useful in support of PK-ADME-related studies. With conventional tissue biopsy as precedent, it is envisioned that researchers will turn to less invasive "liquid biopsy" methods in support of ADME-related studies (e.g., profiling of plasma-derived tissue-specific nanovesicles). Generation of such multi-organ liquid biopsy data, in larger numbers of subjects and at multiple study time points, will provide a rich dataset for modeling purposes.

Introduction

In general, the various processes governing the pharmacokinetics-absorption, distribution, metabolism and excretion (PK-ADME) profiles of drugs and various endogenous compounds are very complex, dynamic and often involve multiple organs such as the gut and liver (Rodrigues and Rowland, 2019). For example, drug interactions can be complicated by a subject's genotype, age, and disease state, and involve the complex interplay of drug-metabolizing enzymes and transporters. Even drug interactions involving a single enzyme, such as cytochrome P450 (CYP) 3A4, can manifest in the gut and liver and present multiple (mixed) mechanisms involving induction, reversible inhibition and mechanism-based inhibition. This has galvanized the development of complex physiologically-based pharmacokinetics (PBPK) models, requiring increased amounts of input data, and has fostered a greater appreciation for the importance of drug interaction time courses (e.g., time to onset versus washout) and the timing of victim drug dosing (versus perpetrator drug).

For the most part, investigators have leveraged in vitro-derived kinetic (substrate and inhibition) data to support in vitro-to-in vivo extrapolations by utilizing vendor-provided human whole cells (e.g., cryopreserved primary hepatocytes) and subcellular tissue fractions (e.g., human liver microsomes). Such in vitro-to-in vivo extrapolation exercises have supported the modeling and prediction of pharmacokinetics (PK), drug interactions, and inter-subject variability. Typically, human whole tissue is obtained from consented deceased single or multi-organ donors (Figure 1). More recently, increased efforts have focused on the development of plate- and chip-based single and multi-organ human microphysiological systems (Wang et al., 2018; Maass et al., 2017). Such systems have garnered the attention of numerous investigators who have grown to

appreciate the need to mimic tissue architecture, dynamic multi-cell type interactions, multi-organ interactions, as well as the supply of nutrients, oxygen and drug substrate under flow conditions.

The above described approaches are patently different from those instances when live study subjects have pre-consented to a single or multiple tissue biopsy procedure in support of drug-metabolizing enzyme and transporter profiling (Padoin et al., 2006; Lefor and Flowers, 1994; Otuya et al., 2018). Alternatively, some investigators have profiled frozen (banked) tissue biopsy samples (Supplemental Figure S1) obtained from subjects who had previously undergone endoscopic, laparoscopic, or open surgery (e.g., bariatric surgery, gall bladder surgery, organ transplant, or the excision of tumor tissue and adjacent non-tumor tissue), or had required biopsy as part of their diagnosis (e.g., ulcerative colitis, non-alcoholic steatohepatitis (NASH), Rotor syndrome, Crohn's disease, Dubin-Johnson Syndrome, and drug-induced liver injury).

Advantages, Disadvantages, and Challenges of Tissue Biopsy

As summarized in Table 1, one of the major advantages of the tissue biopsy approach is that one can dose a subject with a drug, obtain the tissue biopsy, and prepare whole cells and subcellular fractions (e.g., cytosol, 9000g supernatant fraction, microsomes). Thereafter, it is possible to generate an ex vivo drug-metabolizing enzyme and transporter messenger ribonucleic acid (mRNA), microRNA (miR), and protein expression profile, or measure enzyme activity (after the addition of substrate and cofactor) to establish an ex vivo-to-in vivo extrapolation (Supplemental Figure S1).

Once generated, biopsy data can be integrated with PK data, from the same (biopsied) subject or other study subjects, to support PBPK modeling. As discussed in the following sections, there are examples of published reports describing biopsy data to assess CYP3A4 inhibition after dosing of a mechanism-based inhibitor drug, modeling of rifampicin auto-induction by considering the induction of P-glycoprotein (Pgp), as well as studying the impact of *CYP3A5* genotype on PK (Hanke et al., 2018; Quinney et al., 2010; Zhang et al., 2009; Mouly et al., 2005). There are also examples of publications describing the use of tissue biopsy data to rationalize the scaling factors used in PBPK modeling, the study of drug-metabolizing enzyme tissue expression versus plasma-based trait measures (e.g., CYP3A erythromycin breath test and 4β-hydroxycholesterol), and efforts to simply garner mechanistic insight regarding an observed drug interaction (Yamazaki et al., 2019; Lown et al., 1994; Marschall et al., 2005; Greiner et al.,1999; Westphal et al., 2000; Giessmann et al., 2004a; Brueck et al., 2019). Given the utility of tissue biopsy approaches in support of PK-ADME-related studies, therefore, we sought to review the literature and summarize the findings of exemplar manuscripts. Of the publications reviewed, a few are summarized in tabular form (Tables 2 to 5) and only some are described in more detail. Although largely focused on gut and liver biopsy, we were aware of publications describing ADME gene and protein expression profiling of skin, lung and kidney biopsy samples (Thum et al., 2006; Krogstad et al., 2018; Osman-Ponchet et al., 2014; Joy et al., 2007).

As with any method or approach there are downsides when attempting to include tissue biopsy in protocols. In most cases, such protocols require extensive vetting by institutional review boards and a clear rationale articulated in the accompanying inform consent documentation (Table 1). This is particularly true when studying drug interactions in normal healthy volunteers, deploying such subjects as controls (e.g., versus diseased, organ impaired, pediatric or aged

subjects), and when a study necessitates single (multiple tissue) or more than one (single tissue) biopsy. Investigators may also be limited by the amount of tissue that can be obtained in a biopsy and so they assume that such samples are reflective of the target organ as a whole. Investigators may also be challenged by the number of subjects that can be biopsied or have consented to be biopsied. As described in the following, many studies included less than a dozen subjects and investigators have assumed that the data obtained from a small study applies to a larger and more heterogenous population of subjects.

Tissue Biopsy to Support the Study of Drug-Metabolizing Enzyme and Transporter Induction

In various tissues, the expression of drug-metabolizing enzymes and transporters is known to be under the regulation of nuclear hormone receptors, cytokines, transcription factors, miRs, and epigenetic factors (Rodrigues and Rowland, 2019). Therefore, it is recognized that drugs can bring about interactions involving the repression or induction of gene expression and in vitro studies with plated human primary hepatocytes are now routinely conducted to support the generation of different parameters (e.g., maximal induction, IND_{max} or E_{max}; and the concentration rendering half-maximal induction, EC_{50}) as input for modeling and simulation exercises (Almond et al., 2016; Guo et al., 2013). Beyond primary hepatocytes, some investigators have performed in vitro induction studies with different intestinal cell lines (e.g., Caco-2, LS180, LS174T, and T84) as enterocyte surrogates (Oscarson et al., 2007; Weiss et al., 2009; Aiba et al., 2005; Haslam et al., 2008). By contrast, there are few examples of in vitro induction studies using gut and liver

tissue slices (Supplemental Table S1) and only recently has the induction of CYP3A by rifampicin in isolated cryopreserved human primary enterocytes been described (Li et al., 2018).

A review of the literature has provided numerous examples of induction studies employing a tissue biopsy approach (Table 2). In most cases, subjects have consented to pre- and post-treatment biopsies, while some investigators reported data following the single biopsy of parallel groups of subjects (e.g., placebo versus treatment). Studies have encompassed the dosing of drugs (e.g., rifampicin, carbamazepine, efavirenz, omeprazole), smoking, and the consumption of alcohol, natural products (e.g., curcumin), and chargrilled meat. As summarized in Table 2, changes in drug-metabolizing enzyme and transporter mRNA and protein expression in gut and liver biopsies are usually less than 10-fold, a dynamic range more consistent with tissue slice data versus the fold-induction values (IND_{max} or E_{max}) obtained with isolated and plated primary cells (Supplemental Table S1; Almond et al., 2016; Guo et al., 2013). To some investigators, such a magnitude of change is unanticipated, especially when one considers that the concentration of the inducer in the gut during first pass is likely to be very high compared to pregnane X receptor or constitutive androstane receptor EC_{50} values (e.g., >0.1 mM for rifampicin and carbamazepine) (Brueck et al., 2019). This has implications when attempting to predict induction, or complex mixed-mechanism (induction-reversible inhibition-mechanism based inhibition) drug interactions with new chemical entities (NCEs).

Rifampicin and Carbamazepine as Inducers

Profiling of Liver Tissue After Rifampicin. The report by Marschall et al (2005) is particularly important, because it is one of the few examples of liver biopsy following an inducer. The study enrolled thirty healthy gallstone patients scheduled for cholestectomy, which were

randomized to a known pregnane X receptor agonist (rifampicin, 600 mg/day for 1 week),

ursodeoxycholic acid (1 g/day for 3 weeks) or no medication before surgery. A wedge liver biopsy

specimen was taken to study the expression of transporters and drug-metabolizing enzymes. As

expected, the authors determined that CYP3A4 mRNA was induced (3-fold) by rifampicin, which

corresponded to a 247% increase in plasma 4β-hydroxycholesterol levels. In contrast,

ursodeoxycholic acid did not induce CYP3A4 mRNA and there was less impact on plasma 4β-

hydroxycholesterol levels (38% increase). Of note, rifampicin induced liver UDP-

glucuronosyltransferase (UGT) 1A1 (2-fold) and multidrug resistance-associated protein (MRP) 2

(~2-fold) but did not induce organic anion transporting polypeptide (OATP) 1B1 mRNA. To our

knowledge, this is the first study to relate changes in liver (biopsy) CYP3A4 expression to plasma

4β-hydroxycholesterol in the same subjects and to directly assess the induction of OATP in vivo

in human liver.

Profiling of Gut Tissue After Rifampicin and Carbamazepine. We felt it important to

include the report of Brueck et al (2019), as it appeared on line during the writing of this review.

The authors leveraged their access to banked duodenal biopsy samples from subjects who had been

dosed rifampicin (600 mg per day, for 8 days), or carbamazepine (600 mg per day, 14 to 18 days),

and reported the impact of each inducer on pregnane X receptor and constitutive androstane

receptor mRNA expression, fold-changes in both mRNA and protein expression for three ABC

transporters (Pgp, MRP2, and breast cancer resistance protein, [BCRP]), and the expression

profile of numerous miR species. To our knowledge, this is the first report to describe miR

expression profiles in human tissue biopsy samples following an inducer drug. The authors were

able to determine that after rifampicin dosing there was a correlation between Pgp (e.g., versus

miR-485-3p, r = -0.452, p = 0.027) and BCRP (e.g., versus miR-577, r = -0.437, p < 0.033) protein

expression with miR levels. After carbamazepine, a significant correlation was also noted between MRP2 protein and miR26a-5p expression (r = -0.587, p < 0.027). The same authors reported a statistically significant (albeit weak) increase in gut OATP2B1 mRNA expression following rifampicin (1.2-fold) and carbamazepine (1.5-fold). Such a result is in reasonable agreement with the results of Oscarson et al. (2007), who reported no OATP induction in duodenal biopsies of seven subjects following rifampicin (data not shown). In the same subjects, gut CYP3A4, Pgp (ATP-binding cassette (ABC) B1, ABCB1) and MRP2 (ABCC2) mRNA expression was increased (~2- to 3-fold). Compared to rifampicin, carbamazepine presents as a better inducer of OATPs, since Oscarson et al (2006) described induction of OATP1B1 (~1.7-fold), OATP1B3 (~2-fold) and OATP2B1 (~1.3-fold) mRNA in the livers of two epileptic patients treated with the drug. Although some investigators have evoked an E_{max} ~2 for OATP1B induction by rifampicin to support their PBPK modeling effort, direct evidence for such changes in OATP expression in vivo remains elusive (Asaumi et al., 2019; Marschall et al., 2005; Oscarson et al., 2007; Brueck et al., 2019).

Study of CYP2D6 Induction. Rifampicin is regarded as a pleiotropic inducer (Rae et al., 2001), so it is not surprising that there are numerous reports describing its impact on drug-metabolizing enzyme and transporter expression in human gut and liver (Table 2). However, it appears that not every CYP form is induced. For example, Glaser et al (2005) reported no statistically significant induction of CYP2D6 protein in preparations of gut biopsy-derived shed enterocytes following rifampicin dosing; CYP3A4 protein expression was increased (3.3-fold) in the same individuals. Such data are consistent with the results obtained with a humanized mouse model expressing human pregnane X receptor, CYP2D6 and CYP3A4 in the gut and liver (Scheer et al., 2015); following rifampicin, CYP3A4 was induced but no induction of gut or liver CYP2D6

was evident. Unfortunately, there are no human biopsy data describing the impact of rifampicin or other inducers on CYP2D6 expression in the liver, although in vitro data (e.g., human liver slices with rifampicin) present no induction (Supplemental Table S1). Such results are important, because it is accepted that often used CYP2D6 phenotyping tools (e.g., dextrorphan-to-dextromethorphan concentration ratio in urine and plasma) are contaminated by CYP3A and changes in metabolite-to-parent ratio after an inducer could be incorrectly ascribed to CYP2D6 (Jones et al., 1996; Gorski et al., 1994; Schmider et al., 1997). Consequently, in the absence of tissue biopsy data, there is no direct clinical evidence for the induction (or repression) of CYP2D6 expression (Pan et al., 2017; Sage et al., 2017). In this regard, Ke et al (2013) have used a PBPK modeling approach to assess the impact of pregnancy on the PK of various CYP2D6 substrates including dextromethorphan (e.g., metoprolol and clonidine). They concluded that its expression would have to be induced ~2-fold to account for the observed changes in PK. Although challenging, tissue biopsy profiling would be the most useful approach to directly determine whether such a 2-fold increase in CYP2D6 expression is evident in pregnant women.

Profiling of Pgp Expression in Gut Biopsies to Reconcile Observed Drug Interaction.
We are aware of at least two reports describing Pgp expression profiling of gut biopsies to investigate drug interactions with rifampicin. One study published by Greiner et al (1999) described the impact of rifampicin on digoxin PK. The authors had hypothesized that concomitant rifampicin therapy may affect digoxin disposition in humans by inducing Pgp. They compared the single-dose PK of digoxin (1 mg oral and 1 mg intravenous) before and after co-administration of rifampicin (600 mg/day for 10 days). Duodenal biopsies were also obtained from each study subject before and after the administration of rifampicin. The investigators described that although the exposure of digoxin decreased with rifampicin, its renal clearance and half-life were not

altered. Importantly, it was shown that rifampicin increased intestinal Pgp protein expression ~3.5-fold. Similarly, Westphal et al (2000) studied the PK of talinolol before and after co-administration of rifampicin (600 mg per day for 9 days). During rifampicin treatment, the areas under the curve of intravenous and oral talinolol were significantly lower (21% and 35%; P < 0.05). In this study, it was also shown that rifampicin treatment resulted in a significant increase in Pgp expression in duodenal biopsies (protein, 4.2-fold; and mRNA, 2.4-fold). Such data are very useful because they can support PBPK modeling. This is exemplified by the recent publication of Yamazaki et al (2019), who modeled the effect of rifampicin on the PK of 4 different Pgp substrates (digoxin, talinolol, quinidine and dabigatran etexilate). The authors obtained scaling factors for their in vitro-derived Pgp kinetic data, focused on the maximal rate of flux (J_{max}), to adequately recover the clinically observed results. Consistent with published gut biopsy data, they demonstrated that their model could recover the clinically observed drug interaction with rifampicin if Pgp expression in the gut was induced 3- to 4-fold.

Efavirenz as Inducer

Reports by Oswald et al (2012) and Mouly et al (2002) also illustrate the usefulness of tissue biopsy data to rationalize drug interactions. Both sets of investigators were able to assess efavirenz, a non-nucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection, as an inducer and confirmed that its impact on plasma-based PK trait measures was driven by induction of CYP3A4 in the liver and not the intestine. In fact, efavirenz was shown not to induce CYP3A4, CYP2B6, Pgp (ABCB1), OATP2B1 (SLCO2B1) or UGT1A1 mRNA in duodenal tissue biopsies (Oswald et al., 2012). The exact reason for the observed tissue specific induction is not known and for certain NCEs in development such results could cast doubt on the use of hepatocyte-based in vitro data to predict induction in the gut.

Atorvastatin as Inducer

Although rifampicin does not appear to induce OATPs in vivo, Bjorkem-Bergman et al (2013) did report that OATP2B1 (solute carrier (SLC) O2B1) mRNA was statistically significantly induced (3-fold; P < 0.05) in liver biopsies of subjects receiving atorvastatin (80 mg for 4 weeks). In the same study, a ~2-fold induction of liver Pgp (ABCB1) and breast cancer resistance protein (BCRP, ABCG2) was noted in the absence of induction of OATP1B1 and CYP3A4 mRNA. Interestingly, fluvastatin (20 mg/day for 4 weeks) elicited a relatively minimal effect on hepatic gene expression. The exact mechanism of how atorvastatin brings about induction of hepatic OATP2B1 in vivo is not known and needs confirmation.

Impact of Smoking on CYP1A Tissue Expression

Tissue biopsy approaches are not limited to the study of drug-metabolizing enzyme or transporter induction by drugs. For example, Buchthal et al (1995) and Pelkonen et al (1986) were able to leverage tissue biopsy samples to measure CYP1A-catalyzed 7-ethoxyresorufin *O*-deethylase activity in smokers. Buchthal et al (1995) obtained duodenal biopsies (20 smokers, 21 nonsmokers, and 10 nonsmokers receiving omeprazole 20 to 60 mg/day for at least 1 week) and demonstrated a 4.5-fold and 2.5-fold increase in activity in smokers and omeprazole-dosed non-smokers, respectively. In agreement, Pelkonen et al (1986) also reported a 3.3-fold increase in 7-ethoxyresorufin *O*-deethylase activity in the liver biopsies of smokers. Such data complement the results of other studies showing that cigarette smoking induces CYP1A, in addition to CYP1B1, in lungs (Kim et al., 2004; Smith et al., 2001; Thum et al., 2006). Evidently, cigarette smoking induces CYP1A expression and activity in at least three organs.

Tissue Biopsy to Study CYP Inhibition

CYP3A4 expressed in the intestine and liver is also subjected to inhibition by drug interaction perpetrators. In the past, assessment of CYP3A4 inhibition has involved both intravenous and oral administration of a probe drug (e.g., midazolam) and the measurement of changes in its plasma AUC (area under the drug concentration versus time curve) or the metabolite-to-parent concentration ratio (e.g., 1'-hydroxymidazolam-to-midazolam ratio) in plasma (Gorski et al., 1998). It has been assumed that the clearance of an intravenously-dosed probe drug is largely reflective of changes in liver CYP3A4 activity. Therefore, deconvolution of oral versus intravenous probe drug plasma AUC ratios (inhibitor versus placebo) can provide information regarding the inhibition of intestinal CYP3A4. In this regard, widely used CYP3A biomarkers (e.g., plasma 4β-hydroxcholesterol and urinary 6β-hydroxycortisol-to-cortisol ratio) may not be useful and investigators have leveraged a gut biopsy approach (Rodrigues and Rowland, 2019).

Clarithromycin

We are aware of at least three studies describing the biopsy of subjects dosed with a known CYP3A4 inhibitor (Table 3). Two studies described duodenal biopsies obtained pre- and post-dosing with clarithromycin 500 mg twice a day for about one week (Pinto et al., 2005a; Quinney et al., 2013). In both cases, gut homogenate was prepared and a decrease (64 to 74%) in measured midazolam 1'-hydroxylase activity was reported. Clarithromycin is known to be a mechanism-based inhibitor of CYP3A4 and forms a macrolide (CYP-iron-nitrosoalkane) complex with the enzyme. Interestingly, the measured decrease in midazolam 1'-hydroylase activity agreed well with PBPK model-based estimates of CYP3A inhibition in the gut by clarithromycin (Quinney et al., 2010). Presumably the complex was stable enough to survive the tissue biopsy and

homogenization process. Both groups also assessed the inhibition of hepatic CYP3A4 by determining the 1'-hydroxymidazolam-to-midazolam concentration ratio in plasma (3hr) after intravenous midazolam. Of note, clarithromycin had a minimal impact on CYP3A4 protein expression in the gut. In one of the studies (Pinto et al., 2005a), the authors measured the concentration of clarithromycin in the gut homogenate (0.42 to 2.4 nmol/L) and serum (~4 µM).

Diltiazem

Likewise, Pinto et al (2005b) studied diltiazem (100 mg twice daily for 7 days), also a CYP3A4 complex former, as a CYP3A4 inhibitor and reported a 62% decrease in gut biopsy homogenate-mediated midazolam 1'-hydroylase activity (Table 3). There was no significant impact on gut biopsy CYP3A4 mRNA and protein expression. As in the case of clarithromycin, the authors were able to relate these biopsy-generated data to a PBPK model-based prediction of gut wall CYP3A4 inhibition by diltiazem (Zhang et al., 2009). To date, we are not aware of any reports describing liver biopsy following the administration of agents such as clarithromycin and diltiazem.

Tissue Biopsy to Study the Impact of Disease, Age, and Genotype on the Expression and Activity of Drug-Metabolizing Enzymes and Transporters

Tissue biopsy samples have also supported various efforts to study the impact of disease on the expression of drug-metabolizing enzymes and transporters; diseases have included ulcerative colitis, obesity, celiac, Crohn's, and NASH (Table 4). Although not tabulated, it is worth mentioning that at least two groups have profiled tissue biopsies of virus-infected subjects.

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In the first example, Kis et al (2016) completed extensive (microarray) gene expression profiling of CYPs, nuclear hormone receptors, SLCs, ABC transporters, and markers of inflammation in jejunal mucosal biopsies of subjects that had been diagnosed as human immunodeficiency virus type 1 positive and were either antiretroviral therapy active (plasma viral load > 5,000 copies/mL) or naïve (plasma viral load <50 copies/mL). The study also encompassed immunohistochemical analysis of CYP3A4, Pgp, BCRP and MRP2. Similarly, Nakai et al (2008) were able to characterize the hepatic expression profiles (CYPs, ABC transporters, SLCs, nuclear hormone receptors, and proinflammatory cytokines) of biopsied subjects infected with hepatitis C, who had been subdivided into different stages or fibrosis, and the results were compared to liver tissue of non-infected subjects.

In most cases, investigators have leveraged the availability of tissue resulting from surgery or tissue biopsy as part of direct confirmative diagnosis. Beyond diseased subjects, tissue biopsy has also been deployed to study the effect of age on ADME gene and protein expression, as well as genotype-phenotype associations.

Disease

Intestine. Miyauchi et al (2016) were able to apply quantitative (targeted) tandem liquid chromatography-mass spectrometry (LC/MS)-based proteomics to determine the expression levels of drug-metabolizing enzymes and transporters in human jejunal tissues excised from morbidly obese subjects during gastric bypass surgery. Protein expression levels of 15 different CYP forms, 10 UGTs, CYP reductase, and 49 transporters were determined. Likewise, Erdmann et al (2019) obtained gut biopsy specimens from the inflamed and noninflamed tissues of 10 patients with ulcerative colitis as well as colonic control tissues of 10 patients without inflammation. Levels of

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both protein and mRNA expression were quantified and the authors concluded that some enzymes (e.g., CYP2C9, UGT1A1) and transporters (e.g., monocarboxylate transporter 1, Pgp and BCRP) were significantly decreased during inflammation. Englund et al (2007) similarly reported a decrease (vs normal controls) in BCRP (ABCG2) mRNA in the colon (89% decrease) and rectal (84% decrease) biopsies of patients with active ulcerative colitis. Expression of ABCB1 (Pgp) mRNA was also decreased in the colon (78% decrease) and rectal (66% decrease) biopsies of the same patients. For both ABC transporters, the decrease in mRNA expression was consistent with protein expression changes (immunoblotting) and the effect was less pronounced in biopsy samples of subjects that were in disease remission. The same authors noted that ABCC2 (MRP2) mRNA expression was not impacted by the disease. Importantly, the authors were able to measure the expression of various proinflammatory cytokines (CD45, interleukin 1β, and interleukin 6) in the same biopsy samples and noted that they were markedly elevated ($\geq$4-fold) in the patients with active disease versus control subjects and patients under remission. The above findings are consistent with the recently published results of Wilson et al (2019), who showed that there is a decrease in CYP3A4 (78%) and Pgp (85%) protein expression in colon biopsies of patients with Crohn's disease.

Even in the absence of mRNA quantitation and LC/MS-based proteomics, Lang et al (1996) were able to use formalin-fixed jejunal biopsy specimens, from patients with celiac disease at variable times before and after treatment with a gluten-free diet, for immunoperoxidase staining after incubation with anti-CYP3A4 antisera. Based on the intensity of staining in individual enterocytes, as well as the total number of enterocytes stained, the authors concluded that patients with celiac disease presented lower intestinal CYP3A immunoreactivity and that treatment with a gluten-free diet is associated with an increase in intestinal CYP3A protein.

Liver. With the increased interest in NASH, and its potential impact on the PK-ADME profiles of drugs, we noted the report by Woolsey et al (2015). The authors examined CYP3A activity in healthy volunteers, as well as subjects with biopsy-proven NASH, after oral midazolam and measurement of plasma 4β-hydroxycholesterol. Biopsied subjects with NASH presented 2.4-fold higher plasma midazolam levels and a statistically significant 69% decrease in hepatic CYP3A4 mRNA expression (versus controls). The same authors also reported that plasma 4β-hydroxycholesterol was 51% and 37% lower (versus control subjects) in subjects with simple steatosis and NASH, respectively. Collectively, plasma-based trait measures agreed with liver biopsy data in this study.

Ontogeny

In an extensive investigation, Fakhoury et al (2005) were able to obtain a total of 59 normal duodenal biopsies from children aged 1 month to 17 years (divided into 3 groups; 1 month to 1 yr; 1 to 6 yrs; and > 6yrs). The authors deployed both immunohistochemistry and mRNA quantification. In this instance, CYP3A protein was detected in all enterocytes in the samples from patients over 6 months of age, but not in biopsy samples of younger subjects. Pgp protein was also detectable on the surfaces of the enterocytes. Both CYP3A and Pgp mRNA levels were highly variable, but the authors reported that CYP3A4 and CYP3A5 mRNA levels were high during the first year of life and decreased with age. It was concluded that neonates and infants presented significant expression of CYP3A and Pgp mRNA in the intestine, suggesting a different maturation profile of CYP3A and Pgp with age in the liver versus intestine. The approach is very different from other investigators, who have used tissue from deceased donors (Mooij et al., 2014), and relates to ongoing efforts to more holistically model the oral PK of CYP3A substrates by considering age-related changes in both intestinal and hepatic extraction (Brussee et al., 2018).

Relating Genotype to Phenotype

It is well established that numerous human CYP forms, such as CYP2D6, CYP2C19, and CYP2C9, are expressed polymorphically and efforts have focused on relating genotype to their protein expression in the human liver (Weiß et al., 2018). Such efforts are important, because the correlation of genotype with plasma-based phenotypic trait measures is not always apparent (Waring, 2019). In this regard, the work of McConnachie et al (2004) is unique, because the authors included tissue biopsy specimens collected from genotyped control subjects with no apparent liver disease. CYP2D6 activity in the homogenized biopsy samples was measured using a novel selective high-clearance substrate (R-568 metabolite M1 formation) and the liver samples were genotyped for the 6 most common *CYP2D6* genetic variants (*3, *4, *5, *6, *7, and *8). The authors determined that the improved assay sensitivity supported the evaluation of CYP2D6 enzyme activity in a few milligrams of liver tissue collected from the biopsy specimens. This meant that they could determine that CYP2D6 activity was decreased ($\geq$66% versus n = 7 *CYP2D6*1/*1* subjects) in the livers of four subjects carrying the *4 and/or *5 allele. The same individuals also presented a greater than 90% decrease in CYP2D6 mRNA expression versus the reference subjects.

Studies Describing Multi-Organ Biopsies

Gut and Liver

Relatively few reports describe subjects undergoing multiple organ biopsies (Table 5). For example, Von Richter et al (2004) were able to obtain duodenum, proximal jejunum, and liver

wedge biopsy specimens from 15 patients undergoing a gastrointestinal operation. Enterocytes were isolated from the intestinal samples. After homogenization, the expression of CYP3A4, CYP3A5, and Pgp in gut and liver was determined. *CYP3A5* genotype was considered also and two subjects (*CYP3A5*1/*3*) expressed quantifiable hepatic CYP3A5, unlike the remaining subjects who were genotyped *CYP3A5*3/*3* and did not express detectable CYP3A5. Of note, no intraindividual correlations were evident between the intestine and liver with respect to CYP3A4 expression, Pgp expression, or measured CYP3A4 catalytic activities (verapamil *N*-dealkylation and *N*-demethylation).

Similarly, Uvestad et al (2013) investigated the correlation between gut and liver OATP1B1, Pgp, and CYP3A4 expression and the PK of atorvastatin. In this instance, 21 obese patients were consented for paired biopsies (liver and intestinal segments) and *SLCO1B1* genotyping. The authors reported that ~30% of the variation in oral clearance of atorvastatin could be ascribed to hepatic OATP1B1 protein expression. Subjects carrying the *SLCO1B1* c.521C variant allele exhibited 45% lower atorvastatin oral clearance and there was no association between the hepatic and intestinal expression of Pgp and CYP3A4 and the PK of atorvastatin.

At the time of writing, were became aware of the report by Krogstad et al (2019). The authors were able to obtain matched liver and jejunal biopsy samples from CYP genotyped patients with obesity (n = 20 subjects). Both gut and liver microsomes were prepared and various CYP activities were measured (CYP3A-dependent midazolam 1-hydroxylase, CYP2D6-dependent bufuralol 1-hydroxylase, CYP2C19-dependent mephenytoin 4-hydroxylase, CYP2C9-dependent diclofenac 4-hydroxylase, CYP2C8-dependent amodiaquine *N*-demethylase, CYP2B6-dependent bupropion 6-hydroxylase, and CYP1A2-dependent phenacetin *O*-deethylase). CYP activity in each gut microsomal preparation was compared to its matched liver counterpart and it was possible

to obtain correlation coefficients for each CYP form (gut vs liver) and across CYP forms (liver and gut). Because the subjects were genotyped, it was also possible to compare liver (e.g., *CYP1A2*1/*1* vs *CYP1A2*1F/*1F*, *CYP2C9*1/*1* vs *CYP2C9*1/*2*, *CYP2C19*1/*1* vs *CYP2C19*1/*2*, and *CYP2D6*1/*1* vs *CYP2D6*5/*5*) and gut (e.g., *CYP2D6*1/*1* vs *CYP2D6*5/*5* and *CYP2C9*1/*1* vs *CYP2C9*1/*2*) microsomal CYP activity for subjects carrying different alleles.

Multiple Intestinal Segments

We are aware of two groups that have described tissue profiling of different human intestine segments. Focused on studying the gut expression of four human CYPs (CYP2C8, CYP2E1, CYP3A4, and CYP3A5), Bergheim et al (2005) were able to obtain biopsies from the ascending, descending and sigmoid colon of test subjects. Although extensive interindividual variability was found for the expression of the four CYPs, the authors concluded that the expression of CYP2C8 in the ascending colon (versus the sigmoid colon) and contrasted it with CYP2E1 and CYP3A5 mRNA expression. Likewise, Meier et al (2007) were able to consent 10 healthy subjects to biopsies of five intestinal segments; duodenum, ileum, ascending colon, transverse colon, and descending colon. The samples were subjected to mRNA expression profiling and it was possible to obtain data regarding the regional distribution of 15 different SLCs along the human intestinal tract. It was found that some of the SLCs were more of less expressed in all gut segments (e.g., OATP2B1, organic zwitterion/cation transporters 1 and 2, and equilibrative nucleoside transporter 1), while others were more highly expressed in the duodenum and ileum (e.g., apical sodium-dependent bile acid transporter, peptide transporter 1, serotonin transporter, and concentrative nucleoside transporters 1 and 2).

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Advent of Liquid Biopsy

To date, the concept of liquid biopsy, which involves the profiling of biofluids for solid organ- or tumor-specific markers, has gained traction in the field of oncology (Mader and Pantel, 2017). However, as previously described, it is envisioned that liquid biopsy methods will also be applied in support of ADME-related studies (Rodrigues and Rowland, 2019). For example, liquid biopsy would involve the use of validated methods to obtain global or tissue-specific preparations of cargo-laden (e.g., protein, mRNA, miR) nanovesicles (exosomes) from human blood (Figure 1; Supplemental Figure S1 and S2). Upon isolation, nanovesicle cargo would be profiled for drug-metabolizing enzyme expression (mRNA, protein) and activity.   Likewise, SLC and ABC transporter expression could be determined. As in the case of conventional tissue biopsy samples, liquid biopsy samples could support both ex vivo (e.g., pre- versus post-drug) and in vitro studies. Generation of such multi-organ liquid biopsy data in larger numbers of subjects, and at multiple time points in a Phase 1 study, would provide a richer dataset to support the PBPK modeling of NCEs. Importantly, the ability to obtained preparations of exosomes from blood and urine will greatly facilitate protocol review and study subject consenting (Table 1).

As described by Rodrigues and Rowland (2019) and summarized in Table 1, there are some challenges when it comes to the deployment of exosome-based liquid biopsy methods. For example, most researchers are not familiar with exosome isolation protocols (e.g., size exclusion, precipitation, membrane affinity, and immunocapture), as well as exosome characterization, handling and storage. In particular, because of extracellular vesicle heterogeneity in various biofluids, preparations of exosomes require characterization and verification by microscopic methods (e.g., transmission electron microscopy), size analysis (e.g., nanoparticle tracking; $\geq$30 to ~100 nm diameter) and immuno-quantitation of exosome-enriched marker proteins (e.g., tumor

susceptibility gene 101 programmed cell death 6-interacting protein, and cluster of differentiation 63, 9, and 81). In addition, the purity of the exosome preparation needs to be defined (e.g., ratio of particle number-to-total protein (e.g., >3 x 10^{10} particles per µg protein). Beyond exosome sample characterization, handling and storage, presently it is not known how circulating populations of exosomes relate to their organ of origin, or how data obtained with exosomes are integrated into PBPK models (Table 1). Despite the challenges, exosome-based liquid biopsy approaches have great potential and some examples of their application are described in the following.

Literature Examples Describing the Use of an Exosome-Based Liquid Biopsy Approach

Profiling of Human Blood-Derived Global Exosomes to Study Induction of CYP3A4 After Rifampicin Dosing. Rowland et al (2019) were able to deploy a commercially available membrane affinity spin column kit to isolate total plasma exosomes (transmission electron microscopy, nanoparticle tracking, and tumor susceptibility gene 101 qualified) from rifampicin-dosed (300 mg QD x 7 days) subjects (n = 6). The same subjects also received oral midazolam (1 mg) pre- and post-rifampicin. For the first time, it was reported that exosomal CYP3A4 expression (mRNA and protein) is well correlated with the nicotinamide adenine dinucleotide phosphate-dependent exosomal CYP3A4 activity (midazolam 1-hydroxylase) following the addition of a pore forming agent (alamethicin). Importantly, rifampicin brought about an increase in exosomal CYP3A4 mRNA expression (up to 41-fold), protein expression (up to 3.5-fold) and activity (up to 2.4-fold). An excellent correlation between the changes in exosomal CYP3A4 mRNA (r2 = 0.882), protein (r2 = 0.917), and activity (r2 = 0.828) with midazolam oral clearance was also reported. In addition, exosomal midazolam 1-hydroxylase activity was inhibited (~95%) by the

established CYP3A4 inhibitor CYP3cide (1-methyl-3-[1-methyl-5-(4-methylphenyl)-1H-pyrazol-4-yl]-4-[(3S)-3-piperidin-1-ylpyrrolidin-1-yl]-1H-pyrazolo[3,4-d]pyrimidine). The same exosome preparations were also subjected to targeted proteomic analysis and peptides for numerous additional human CYPs (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A5), CYP reductase, and UGTs (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, and UGT2B15) were detected. For the latter, exosomes were fortified with cofactor (uridine 5'-diphosphoglucuronic acid) and 4-methylumbelliferone glucuronidation was measured with a single K_m (concentration of 4-methylumbelliferone rendering half-maximal rate of glucuronidation) similar to that obtained for cofactor-fortified and alamethicin-treated human liver microsomes. It is envisioned that preparations of plasma-derived global exosomes, such as the ones described above, could be further subjected to immunocapture protocols to enable the isolation of tissue-specific exosomes. The development of such protocols will be important for drug-metabolizing enzymes that reside in both the gut and liver (e.g., CYP3A4).

Human Plasma-Derived Intestinal Exosomes to Support BCRP Expression Profiling. As described by Gotanda et al (2016), it is possible to correlate the expression of intestinal miR-328, which modulates BCRP expression, and the AUC of an orally dosed BCRP substrate (sulfasalazine) across 33 different subjects. The authors come to such a conclusion because they were able to isolate antigen 33 (A33)-enriched plasma exosomes using an anti-A33 antibody immunocapture approach. Compared to other human tissues, A33 is highly expressed in intestinal epithelial cells. Therefore, intestinal epithelial cell-derived plasma exosomes are expected to express high levels of surface A33. Following their immunocapture procedure, the authors confirmed the isolation of exosomes (immunoblotting with antibodies to cluster of differentiation

9 and A33) and showed that their preparations presented elevated expression of two miRs known to be enriched in the human intestine (miR-192 and miR-215 versus liver selective miR-122). Subsequently, the authors described a correlation (r = 0.346, P < 0.049) between the plasma AUC of sulfasalazine and miR-328 expression levels in their gut-derived exosome preparations. Of note, a weaker correlation (r = 0.157, P = 0.328) was obtained for plasma-derived (global) exosomes prior to immunocapture. In the future, it is envisioned that such a study would incorporate *ABCG2* genotype, exosome BCRP protein expression, and a consideration of hepatic miR-328 expression and its impact on the AUC of sulfasalazine following an oral dose.

Isolation of Global Exosomes from Human Urine to Support SLC22A5 Activity Profiling. Console et al (2018) were able to prepare exosomes from human urine to support in vitro transporter studies with carnitine/organic cation transporter 2 (SLC22A5). SLC22A5 is known to mediate the sodium-dependent transport of carnitine and is highly expressed on the apical membranes of renal proximal tubule epithelial cells. In this instance, the authors isolated exosomes from a urine sample by ultracentrifugation and confirmed their identity by immunoblotting with an antibody to tumor susceptibility gene 101 and cluster of differentiation 9. The authors were also able to detect SLC22A5 expression by immunoblotting, reconstitute the isolated exosomes with proteoliposomes, and measure uptake of radiolabeled carnitine in the presence of sodium. Because exosomes are largely formed via an intracellular budding process, involving multivesicular bodies followed by exocytosis, it is assumed that urinary exosomes are reflective of the apical membranes of renal proximal tubule epithelial cells and enriched for SLC22A5. Whether or not plasma-derived exosomes contain functional basolateral kidney (organic anion or cation) and liver (e.g., OATP) transporters is not known.

Conclusions

Based on a review of the literature, it is evident that there are numerous examples of clinical studies leveraging tissue biopsy, albeit with relatively small numbers of subjects, to support the study of drug metabolism, transport, and PK. It is apparent that tissue biopsy data can be useful when testing hypotheses, conducting modeling and simulation exercises, assessing drug interactions, studying phenotype-genotype associations, as well as evaluating the impact of disease and ontogeny on the tissue expression and activity profiles of different drug-metabolizing enzymes and transporters.

Increasingly, however, there will likely be greater demand for multiple-single tissue-biopsies to study drug interaction time courses, the impact of disease over time, or drug dose level-dependency in a single individual. With growing interest in remote sensing (inter-organ communication), and how multi-organ axes drive endogenous compound (endobiotic) PK-ADME processes, govern homeostasis, and respond to disease and drug interactions, it is highly likely that the demand for multi-organ biopsies will increase also (Rodrigues and Rowland, 2019; Momper and Nigam, 2018; Bush et al., 2017; Fu and Cui, 2017; Wu et al., 2011). Ethically, this would prove challenging when studying subjects that do not classify as "normal healthy volunteers" (e.g., diseased, organ impaired, pediatric, elderly and pregnant women). To meet such a need, it is envisioned that PK-ADME researchers, PBPK modelers and clinical pharmacologists will turn to "liquid biopsy" approaches (Rowland et al., 2019; Rodrigues and Rowland, 2019). In the long run, the greater availability of multi-organ derived, banked and profiled liquid biopsy samples from large numbers of individuals, representative of different populations, could greatly support the translation of PK-ADME (Phase 1 study) data obtained with young male normal healthy volunteers.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Rodrigues AD, Rowland. A

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Figure Legends

Fig. 1.   Summary of approaches to obtain human tissue for the support of drug-metabolizing enzyme and transporter profiling

Tissue harvest involves the removal of the entire organ from the donor (usually deceased) and the samples are processed for the preparation of cultured primary cells and various subcellular fractions to support profiling (e.g., mRNA and protein expression, drug-metabolizing enzyme activity). Tissue biopsy involves obtaining a piece of tissue from a live donor (e.g., during surgery or via a scope-guided procedure). With enough sample, it is possible to obtain preparations of whole cells or derive subcellular fractions for profiling. Liquid biopsy involves the isolation of nanovesicles (exosomes) from blood. In this instance, human blood-derived plasma is subjected to a global exosome isolation procedure (e.g., size exclusion chromatography, SEC, or precipitation). The preparation of global exosomes can be profiled directly or further subjected to immunocapture protocols that render tissue-selective exosomes for profiling.

Supplemental Materials

Fig. S1.   Summary of in vitro, ex vivo, and in vivo ADME profiling, and extrapolations to

in vivo (in vitro-to-in vivo, ex vivo-to-in vivo)

Fig. S2.   Differentiating the isolation of nanovesicles (exosomes) from isolated tissue-

derived cells versus immunocaptured tissue-specific exosomes from human plasma

as liquid biopsy

Table S1.   Summary of literature examples describing drug-metabolizing enzyme and

transporter profiling of human tissue slices after incubation with inducer

Table 1

Advantages, disadvantages and challenges of conventional tissue biopsy compared to emerging liquid biopsy approaches

Biopsy type	Advantages	Disadvantages/Challenges
Conventional biopsy (e.g., scope-guided pinch or needle tissue biopsy)	Direct assessment of drug-metabolizing enzyme and transporter expression in target tissue of interest (mRNA and proteomics) in vivo (e.g., following administration of inducer drug)	Requires invasive procedure to obtain tissue, robust study subject consent and more extensive institutional review board approval (especially for normal healthy volunteers)
	With adequate sample size, one can prepare subcellular fractions to support activity assessment	Often limited to one organ site for biopsy and need to assume that sample is representative of whole organ
	Able to correlate biopsy data with trait measures in the same subject (e.g., drug probe and/or biomarker pharmacokinetics)	Studies involving multiple (single tissue) and single (multiple organ) biopsies can be challenging to approve (especially normal healthy volunteers)
	Able to leverage biopsy-derived data as input to support modeling and simulation (e.g., model validation or input to support prediction)	Often only able to biopsy a small set of study subjects ($N \leq 10$); size of tissue sample may limit characterization
Liquid biopsy (e.g., extracellular vesicles isolated from human urine or blood)	Leverages readily obtained body fluids; considered "non-invasive" and supports more straight forward study subject consent language and institutional review board approval	Methods for the isolation, storage and handling of global and tissue-specific blood and urine-derived extracellular vesicles are not widely known within the PK-ADME community and require validation
	Can obtain liquid biopsy at multiple time points in a study and assess time course to support modeling and simulation exercises	How populations of circulating and urinary extracellular vesicles turnover, their composition, and how both relate to the tissue of origin is not known
	Can more readily obtained liquid biopsy from subjects that are not normal healthy volunteers (e.g., pediatric, diseased, organ impaired, pregnant)	How liquid biopsy-derived data are used as input for modeling and simulation exercises is not well developed or validated
	Can obtain liquid biopsy from larger numbers of subjects to support more robust statistical analyses	

Table 2

Summary of literature examples describing drug-metabolizing enzyme and transporter profiling of human tissue biopsy samples

following an inducer

Inducer	mg Dose (Days)	Tissue Biopsy	Fold-Increase	Measurement	Target(s)	Reference
Rifampicin	600 (7)	Liver (pre- vs post-)	1.5	mRNA	ABCC2 MRP2	Marschall et al., 2005
			2.2	Protein		
			2.0	mRNA	UGT1A1	
			No change	mRNA	SLCO1B1	
			3.0	mRNA	CYP3A4	
Rifampicin	600 (4)	Liver (pre- vs post-)	4.9	Protein	CYP3A4	Ged et al., 1989
Atorvastatin	80 (30)	Liver (Treated vs placebo)	No change	mRNA	SLCO1B1	Bjorkem-Bergman et al., 2013
			3.0	mRNA	SLCO2B1	
			2.4	mRNA	ABCB1	
			2.2	mRNA	ABCG2	
			No change	mRNA	CYP3A4	
Rifampicin	600 (8)	Duodenal (pre- vs post-)	2.0	mRNA	ABCC2 MRP2	Haenisch et al., 2008
			2.1	Protein		
Rifampicin	600 (9)	Duodenal (pre- vs post-)	1.8	mRNA	ABCC2 MRP2	Fromm et al., 2000
			1.4	Protein		
Rifampicin	600 (7)	Duodenal (pre- vs post-)	2.2-4.4	Protein	CYP3A4	Greiner et al., 1999
			1.4-3.5	Protein	Pgp	
Rifampicin	600 (9)	Duodenal (pre- vs post-)	2.4	mRNA	ABCB1	Westphal et al., 2000
			4.2	Protein	Pgp	

Table 2 (Continued)

Inducer	mg Dose (Days)	Tissue Biopsy	Fold-Increase	Measurement	Target(s)	Reference
Rifampicin	600 (10)	Shed Enterocytes (pre- vs post-)	No change	Protein	CYP2D6	Glaeser et al., 2005
			2.0	Protein	CYP2C8	
			1.4	Protein	CYP2C9	
			2.3	mRNA	CYP3A4	
			3.3	Protein		
Rifampicin	600 (9)	Duodenal (pre- vs post-)	3.0	mRNA	ABCB1	Giessmann et al., 2004a
			8.3	Protein	Pgp	
			2.1	mRNA	ABCC2	
			5.7	Protein	MRP2	
Rifampicin	600 (9)	Duodenal (pre- vs post-)	2.2	mRNA	CYP3A4	Oscarson et al., 2007
			2.6	mRNA	ABCB1	
			2.2	mRNA	ABCC2	
			No change	mRNA	OATPs	
Rifampicin	600 (8)	Duodenal (pre- vs post-)	Variable	microRNA	Multiple	Brueck et al., 2019
Carbamazepine	600 (14-18)		Variable	microRNA	Multiple	
Carbamazepine	600 (14-18)	Duodenal (pre- vs post-)	No change	Protein	Pgp ABCB1	Giessmann et al., 2004b
			3.5	mRNA		
			3.5	Protein	MRP2 ABCC2	
			2.5	mRNA		
Efavirenz	400 (9)	Duodenal (pre- vs post-)	No change	mRNA	CYP3A4	Oswald et al., 2012
				mRNA	CYP2B6	
				mRNA	ABCB1	
				mRNA	UGT1A1	
				mRNA	SLCO2B1	

Table 2 (Continued)

Inducer	mg Dose (Days)	Tissue Biopsy	Fold-Increase	Measurement	Target(s)	Reference
Efavirenz	200, 400 (10)	Duodenal (pre- vs post-)	No change	Protein Protein	Pgp CYP3A4	Mouly et al., 2002
Smoking	3-30 cigarettes per day	Duodenal (smoking vs non-smoking)	4.5	Activity[a]	CYP1A1	Buchthal et al., 1995
Omeprazole	20-60 (7)	Duodenal (pre- vs post-)	2.5	Activity[a]		
Smoking	Not Specified	Liver (smoking vs non-smoking)	3.3	Activity[a]	CYP1A1	Pelkonen et al., 1986
Curcumin	4000 (30)	Rectal (pre- vs post-)	No change	Protein	UGT1A10 UGT2B17	Asher et al., 2016
Chargrilled meat diet	Grilled beef (7)	Duodenal (Day 1, 5, and 12)	No change	Protein/mRNA	Pgp (ABCB1)	Fontana et al., 1999
			No change	Protein/mRNA	CYP3A4	
			No change	Protein/mRNA	CYP3A5	
			Increased	Protein/mRNA	CYP1A1	
Alcohol consumption	Not Specified	Liver (active drinkers vs non-drinkers)	2.0	Protein	CYP2E1	Perrot et al., 1989

CYP, cytochrome P450; UGT, UDP-glucuronosyltransferase; Pgp, P-glycoprotein; ABC, ATP-binding cassette transporter; SLC, solute carrier; MRP, multidrug resistance-associated protein.

[a]7-ethoxyresorufin O-deethylase

Table 3

Summary of literature examples describing drug-metabolizing enzyme profiling of human tissue biopsy samples

following an inhibitor

Inhibitor	mg Dose (Days)	Tissue Biopsy	% Inhibition	Measurement	Target	Reference
Clarithromycin	500 q12 hr (7)	Duodenal (pre- vs post-)	No Change 74%	Protein Expression Activity[a]	CYP3A4	Pinto et al., 2005a
Clarithromycin	500 q12 hr (8)	Duodenal (serial)	No Change 64-72%	Protein Expression Activity[a]	CYP3A4	Quinney et al., 2013
Diltiazem	120 q12 hr (7)	Duodenal (placebo vs treated group)	No Change No Change 62%	mRNA Expression Protein Expression Activity[a]	CYP3A4	Pinto et al., 2005b

CYP3A4, cytochrome P450 3A4.

[a]Midazolam 1'-hydroxylase

Table 4

Summary of literature examples describing drug-metabolizing enzyme and transporter profiling of human tissue biopsy samples to study disease, ontogeny and genotype-phenotype associations

Study	Tissue Biopsy	Change	Measurement	Target	Reference
Non-alcoholic steatohepatitis (vs normal)	Liver	1.5-fold increase 1.4-fold increase	Protein Expression Activity[a]	CYP2E1	Varela et al., 2008
Non-alcoholic steatohepatitis (vs normal)	Liver	69% Decrease	mRNA Expression	CYP3A4	Woolsey et al., 2015
Celiac disease (pre/post gluten-free diet)	Jejunum	Increase	Immunostaining	CYP3A4	Lang et al., 1996
Crohn's disease (vs normal)	Colon	78% Decrease 85% Decrease	Protein Expression Protein Expression	CYP3A4 Pgp	Wilson et al., 2019
Cholestatic (vs normal)	Liver	49% decease	mRNA Expression	SLCO1B1 (OATP2)	Zollner et al., 2001
		34% decrease	mRNA Expression	ABCB11 (BSEP)	
		41% decrease	mRNA Expression	SLC10A1 (NTCP)	

Table 4 (Continued)

Study	Tissue Biopsy	Change	Measurement	Target	Reference
Morbid Obesity	Jejunum	Variable	Proteomics	ABC Transporters	Miyauchi et al., 2016
		Variable		SLC Transporters	
		Variable		P450 (various)	
		Variable		UGT (various)	
Ulcerative colitis (vs non-inflamed tissue)	Rectum and colon	Varied	Protein/mRNA Expression	SLC (various)	Erdmann et al., 2019
		Varied	Protein/mRNA Expression	ABC (various)	
		Varied	Protein/mRNA Expression	P450 (various)	
		Varied	Protein/mRNA Expression	UGT (various)	
Subjects with ulcerative colitis (vs normal/control subjects)	Rectum and colon	~70% decrease (vs control)	mRNA Expression	ABCB1	Englund et al., 2007
		~85% decrease (vs control)	mRNA Expression	ABCG2	
		No change	mRNA Expression	ABCC2	

Table 4 (Continued)

Study	Tissue Biopsy	Change	Measurement	Target	Reference
Impact of age (1 month to 1 yr vs 1 to 6 yrs vs > 6 yrs)	Duodenal	Decrease	mRNA Expression	CYP3A4	Fakhoury et al., 2005
		Decrease	mRNA Expression	CYP3A5	
		Not significant	mRNA Expression	CYP3A7	
		Not significant	mRNA Expression	ABCB1	
CYP2D6 genotyped subjects	Liver (heterozygous variant vs wild type)	≥66% Decrease (vs wild type)	mRNA Expression	CYP2D6	McConnachie et al., 2004
		≥91% Decrease (vs wild type)	Activity[b]		
Gut CYP3A5 expression vs saquinavir oral clearance	Duodenal	No positive correlation	Protein Expression	CYP3A5	Mouly et al., 2005
CYP3A expression (vs erythromycin breath test)	Duodenal	Correlation	Protein/mRNA Expression Activity[c]	CYP3A4/5	Lown et al., 1994
ABCC2 genotyped subjects	Duodenal	Genotype	Protein/mRNA Expression	ABCC2	Haenisch et al., 2008

CYP, cytochrome P450; UGT, UDP-glucuronosyltransferase; Pgp, P-glycoprotein; ABC, ATP-binding cassette transporter; SLC, solute carrier; MRP, multidrug resistance-associated protein; OATP, organic anion transporting polypeptide; BSEP, bile salt export pump; NTCP, sodium-taurocholate co-transporting polypeptide. ABCB1 = Pgp; ABCG2 = breast cancer resistance protein; ABCC2 = MRP2.

[a] Chlorzoxazone hydroxylase

[b] R-568 metabolite (M1) formation

[c] Midazolam 1'-hydroxylase

Table 5

Summary of literature examples describing drug-metabolizing enzyme and transporter profiling of multiple tissue biopsy

samples from same subject

Multiple (Matched) Tissue Biopsy	Measurement	ADME Target	Reference
Duodenum, jejunum, and liver	Protein Expression	Pgp	Von Richter et al., 2004
	Protein Expression	CYP3A4	
	Protein Expression	CYP3A5	
	Activity	CYP3A	
Jejunum and liver	Genotype	Multiple CYP	Krogstad et al., 2019
	Activity	Multiple CYP	
Duodenum, jejunum, ileum, and liver	Protein Expression	Pgp	Ulvestad et al., 2013
	Protein Expression	OATP1B1	
	Protein Expression	CYP3A4	
Duodenum, ileum, colon (ascending), colon (transverse), and colon (descending)	mRNA Expression	Multiple SLC transporters	Meier et al., 2007
Ascending vs sigmoid colon	Protein/mRNA Expression	CYP3A4	Bergheim et al., 2005
	Protein/mRNA Expression	CYP3A5	
	Protein/mRNA Expression	CYP2E1	
	Protein/mRNA Expression	CYP2C	

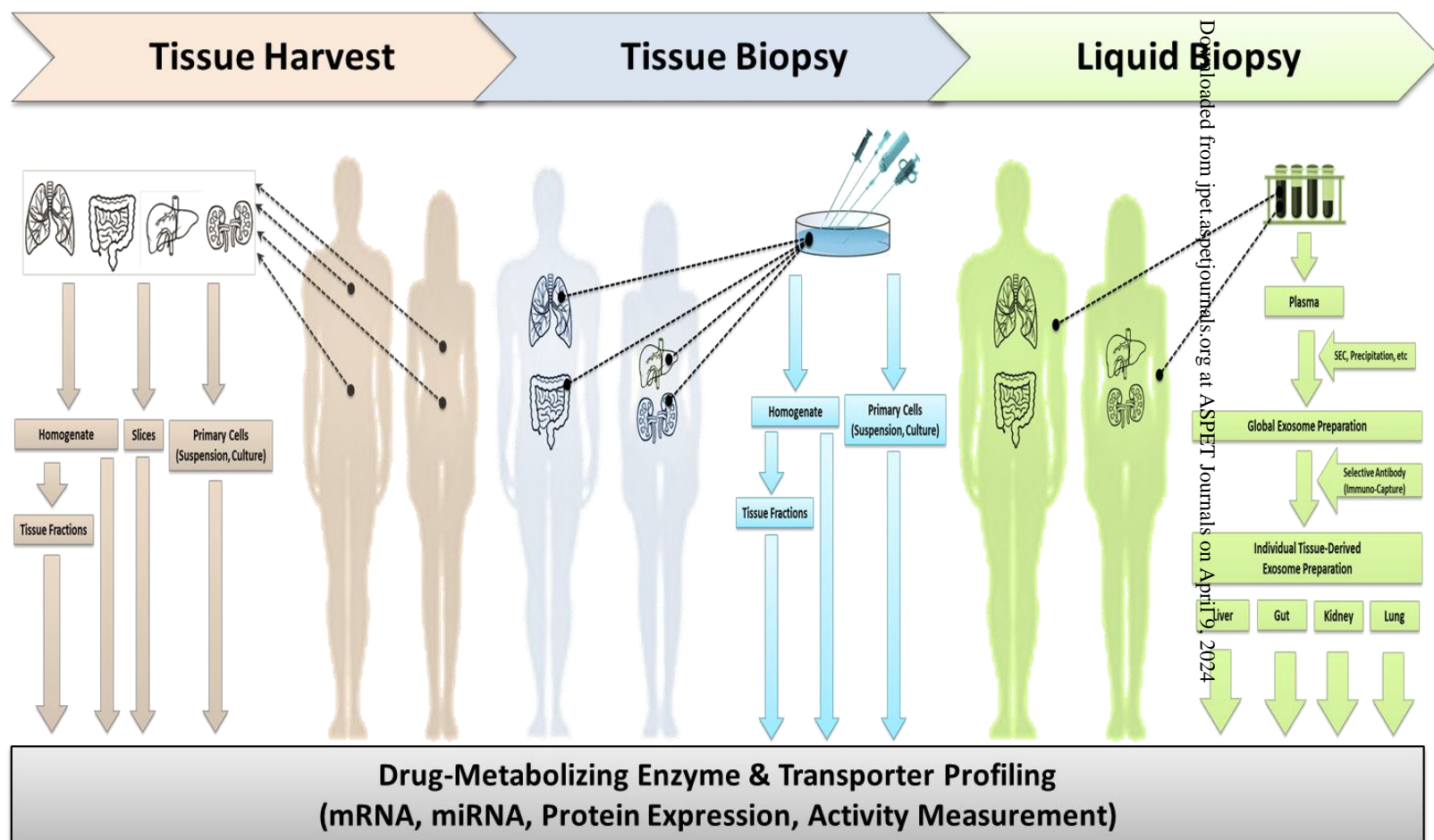CYP, cytochrome P450; Pgp, P-glycoprotein; OATP, organic anion transporting polypeptide; SLC, solute carrier.

Figure 1