Special Section on Pregnancy—Commentary

Drug Metabolism and Transport During Pregnancy: How Does Drug Disposition Change during Pregnancy and What Are the Mechanisms that Cause Such Changes?

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ABSTRACT

There is increasing evidence that pregnancy alters the function of drug-metabolizing enzymes and drug transporters in a gestational-stage and tissue-specific manner. In vivo probe studies have shown that the activity of several hepatic cytochrome P450 enzymes, such as CYP2D6 and CYP3A4, is increased during pregnancy, whereas the activity of others, such as CYP1A2, is decreased. The activity of some renal transporters, including organic cation transporter and P-glycoprotein, also appears to be increased during pregnancy. Although much has been learned, significant gaps still exist in our understanding of the spectrum of drug metabolism and transport genes affected, gestational age–dependent changes in the activity of encoded drug metabolizing and transporting processes, and the mechanisms of pregnancy-induced alterations. In this issue of *Drug Metabolism and Disposition*, a series of articles is presented that address the predictability, mechanisms, and magnitude of changes in drug metabolism and transport processes during pregnancy. The articles highlight state-of-the-art approaches to studying mechanisms of changes in drug disposition during pregnancy, and illustrate the use and integration of data from in vitro models, animal studies, and human clinical studies. The findings presented show the complex inter-relationships between multiple regulators of drug metabolism and transport genes, such as estrogens, progesterone, and growth hormone, and their effects on enzyme and transporter expression in different tissues. The studies provide the impetus for a mechanism- and evidence-based approach to optimally managing drug therapies during pregnancy and improving treatment outcomes.

Introduction

Since the time of the thalidomide tragedy in the late 1950s, and before, there has been a strong reluctance to evaluate the disposition and pharmacological response to drugs in pregnant women. Concerns for the safety of the developing fetus and the mother were behind this de facto policy, resulting, over time, in a dearth of detailed knowledge and pronounced uncertainty about the pharmacokinetic (PK) and pharmacodynamic (PD) behavior of drugs in the pregnant woman. In the absence of evidence, clinicians have been left to treat their pregnant patients empirically and take what guidance they can from treatment recommendations for the nonpregnant woman and a basic understanding of physiologic and biochemical changes that occur during pregnancy.

Pregnancy is associated with many physiologic changes that can influence drug absorption, distribution, metabolism, and excretion, such as an increase in gastric pH and reduction in intestinal motility, increased cardiac output, increased glomerular filtration rate, and reduced plasma albumin concentrations (Anderson, 2005; Feghali and

Mattison, 2011). Although it is not surprising that the pregnant state cannot be represented simply by scalable changes in basic pharmacokinetic parameters (e.g., distribution volume and clearance) that take into account altered physiology, only recently has the research community begun to illuminate some of the profound changes in the biochemistry of drug metabolism and transport that occur during pregnancy. These studies demonstrate the fallacy of many prior assumptions, and the need for expanded research efforts to ensure that the pregnant woman is treated optimally when therapeutic intervention is required. Importantly, as discussed later, there is also emerging evidence for modifications in gene regulation that lead to enhanced and suppressed enzyme/transporter expression and catalytic function.

In the United States, at least 64% of pregnant women take one or more drugs during their pregnancy that are not a vitamin or dietary supplement, and approximately two-thirds of those drugs will never have been formally tested in pregnant women (Glover et al., 2003; Andrade et al., 2004). Information on drug disposition in pregnant women is essential for making rational, evidence-based decisions about drug selection, what dose to use, how frequently to administer the dose, and what level of monitoring is needed to ensure drug safety and efficacy. A pregnant woman is no less likely to need treatment of disease or emergency care than any other woman her age, particularly

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ABBREVIATIONS: EM, extensive metabolizer; FDA, Food and Drug Administration; DMET, drug metabolism and transport; OCT, organic cation transporter; P-gp, P-glycoprotein; P450, cytochrome P450; PBPK, physiologically based pharmacokinetic; PD, pharmacodynamic; PK, pharmacokinetic; UGT, UDP glucuronosyltransferase.

when the untreated chronic (epilepsy, immune disorders, organ transplantation, psychiatric disorders, human immunodeficiency virus infection) or acute (influenza, cancer) conditions can cause real harm to her and possibly the fetus (Little et al., 2009). Moreover, there are serious medical conditions that often emerge as a consequence of pregnancy, such as gestational diabetes, hypertension, and preeclampsia that compel some form of therapeutic intervention, but still lack basic information about drug disposition and response to optimally guide treatment decisions in the patient.

A case in point involves the treatment of gestational diabetes. This is a condition that evolves during pregnancy for reasons that are not completely understood and, if left untreated, is clearly associated with an increased risk of morbidity for the mother and newborn child (Reece and Moore, 2012; Wendland et al., 2012). Until recently, insulin was the only accepted treatment modality because of assurances that the drug would not cross the placenta and directly expose the fetus to its biologic actions, despite the fact that oral hypoglycemics would likely be superior in the management of the condition in the mother (Landon and Gabbe, 2011). To address this issue, National Institutes of Health–funded programs, such as the multicenter Obstetrics-Fetal Pharmacology Research Unit, have been conducting a series of investigations to characterize the disposition of drugs commonly used in nonpregnant women with type II diabetes, such as glyburide and metformin, to identify the optimum treatment regimen for glucose control. Some of the initial data from these investigations demonstrate marked changes during pregnancy in the oral clearance of these drugs—a 100% increase for glyburide (Hebert et al., 2009) and ~50% increase for metformin (Eyal et al., 2010)—that appear to be the result, in part, of increased metabolism (glyburide) or renal secretion (metformin). The exact molecular basis for these catalytic changes remains to be elucidated, but it is clear from PK-PD analysis of the data that control of hyperglycemia is often not optimal when standard drug dosing regimens are used (Hebert et al., 2009).

Another recently described example of altered drug pharmacokinetics during pregnancy involves the immunosuppressant drug tacrolimus (Zheng et al., 2012). In this case, the mean oral clearance of tacrolimus was found to be 39% higher during mid- and late pregnancy, compared with postpartum, which could result in suboptimal blood levels without dose adjustment. Interestingly, the tacrolimus free fraction in blood increased by 91% in the same patients as a consequence of declining hematocrit and albumin concentrations, which, when accompanied by a 45% increase in tacrolimus dose to maintain total blood concentrations, resulted in a doubling of the unbound drug concentrations. Although it is unclear how patient therapy with tacrolimus should be managed during pregnancy (monitor the unbound or total drug concentrations), the marked changes that occur are sobering and illustrative of how physiologic and biochemical changes that occur during pregnancy might profoundly affect drug disposition and response.
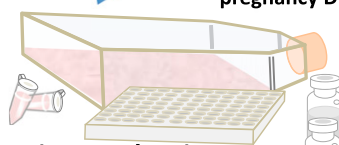
Knowledge on Enzyme-Specific Changes during Pregnancy

Similar to drug-drug interaction studies, the ideal way to understand and predict the changes observed in drug disposition during pregnancy is to obtain sufficient data on probe substrate disposition during gestation that will allow rational extrapolations of how the disposition of specific, clinically relevant drugs is altered during pregnancy (Fig. 1). Such predictions will also allow prioritization of studies conducted in pregnant women to drugs whose disposition is most likely significantly affected by pregnancy. Probe substrate studies are, however, complicated by safety concerns about administering drugs to pregnant women in the absence of clear therapeutic benefit, and the fact that pregnancy is associated with a plethora of physiologic changes that can affect probe disposition in an unpredictable or uncharacterized manner. An approach that appears to be very useful in addressing these issues is physiologically based pharmacokinetic
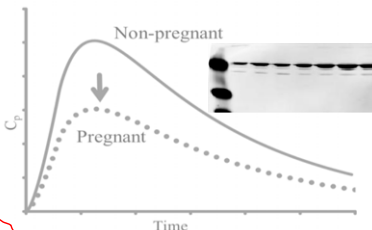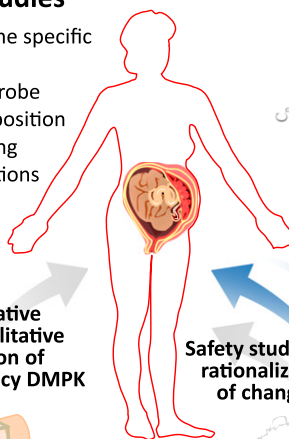

In vivo human clinical studies
- Probe studies to address enzyme specific changes
- PBPK modeling of changes in probe and clinically relevant drug disposition
- Analysis of endogenous signaling molecules and their concentrations throughout gestation
- PBPK models of organ specific mechanisms

Elaboration of mechanisms

Quantitative and qualitative prediction of pregnancy DMPK

Safety studies, rationalization of changes

Correlation of *in vivo* changes and integration of mechanisms

In vitro evaluation
cell lines, hepatocytes and other tissue preparations
- Enzyme specific studies of regulation and existence of induction/down-regulation
- Potency and efficacy of hormones and other regulators
- Enzyme specific mechanisms

Confirmation of mechanisms, and integration with pregnancy

Mechanistic hypotheses

Animal studies
- Determine the effect of pregnancy on specific enzyme and transporter activity/expression
- In vivo mechanistic studies of gene regulation by hormones and other compounds
- Fetal exposure studies and toxicology

Fig. 1. Overview of the integration of in vitro and in vivo data into understanding of drug disposition during pregnancy. DMPK, drug metabolism and pharmacokinetics.

(PBPK) modeling (Abduljalil et al., 2012; Gaohua et al., 2012; Ke et al., 2012). PBPK modeling allows incorporation of physiologic changes together with changes in multiple enzymes into a model that is fitted to best describe the disposition of well characterized drugs. When data from multiple substrates are available, PBPK models can be used to globally validate the presumed changes in specific enzyme activity. This has been done, for example, to model the increase in CYP3A4 activity during pregnancy (Ke et al., 2012), and the results have profound implications to the dosage determination and efficacy of many CYP3A4 substrates during pregnancy, including many of the human immunodeficiency virus protease inhibitors (Roustit et al., 2008). Thus, prior PBPK modeling may enhance the safety of probe studies and improve the interpretation of the resulting PK and PD findings.

Despite the considerable challenges in conducting mechanistically driven pharmacokinetic investigations in pregnant women, an increasing number of studies have been conducted to characterize cytochrome P450 (P450), transporter, and UDP glucuronosyltransferase (UGT) activity during pregnancy. Probe studies that measure P450 enzyme activity during pregnancy are summarized in Table 1. The data in Table 1 were gathered using the U.S. Food and Drug Administration (FDA) recommended list of sensitive markers of P450 enzymes (from the FDA draft guidance of drug-drug interaction studies in 2012) and substrates with a narrow therapeutic index. Although impressive, the table reveals significant gaps in our mechanistic understanding of how activities of all of the key drug-metabolizing P450 enzymes change during pregnancy, and of the time course of changes in enzyme activity during gestation. For example, data on the activity of CYP2C8 and CYP2C19 activity determined using well characterized probes are lacking for any stage of pregnancy. Knowledge of changes in CYP2C9 activity is based on altered phenytoin disposition during pregnancy, which may be confounded by the nonlinear kinetics of phenytoin, altered volume of distribution during pregnancy, and the resulting inter-relationship between volume of distribution changes and clearance changes. Proguanil, a possible CYP2C19 marker, has been studied during pregnancy using single time-point plasma samples. Cycloguanil (metabolite) concentration was decreased by 42% in CYP2C19 extensive metabolizers (EMs), whereas proguanil concentrations were unchanged during pregnancy (McGready et al., 2003). The concentration ratio of proguanil (parent) to cycloguanil (metabolite) concentration ratio increased significantly (by 62%) during pregnancy in CYP2C19 EMs, but it also increased by 40% in CYP2C19 poor metabolizers, suggesting that the change might not be due to altered CYP2C19 activity, but rather altered contributions from other enzymes or the clearance of the metabolite.

Thus, the validity of the proguanil-to-cycloguanil ratio as a CYP2C19-specific marker is not clear, even though the increase in the parent-to-metabolite ratio at a single time point was 3-fold between nonpregnant EMs and poor metabolizers. Interestingly, in vitro, cycloguanil formation was inhibited by troleandomycin (CYP3A4 inhibitor) and furafylline (CYP1A2 inhibitor) (Coller et al., 1999), suggesting that changes in these enzymes as well as in cycloguanil elimination during pregnancy may confound the use of the proguanil-to-cycloguanil ratio as a reliable CYP2C19 biomarker. Together, these analyses suggest that a more complete characterization of the extent of gestational age–dependent changes in CYP2C enzyme activities is needed.

The apparent change in CYP2D6 activity during pregnancy is of special interest, considering the number of drugs that are CYP2D6 substrates and the lack of induction of CYP2D6 by xenobiotics. A relatively early study with metoprolol, a well accepted CYP2D6 probe, included five pregnant women who were not genotyped for CYP2D6. The results of the study showed that oral clearance of the drug was increased approximately 4- to 6-fold (systemic clearance was 25–97% lower postpartum than during pregnancy) (Hogstedt et al., 1985). However, subsequent studies with other CYP2D6 substrates have not demonstrated as impressive an increase in substrate clearance. For example, the urinary ratios of dextromethorphan to dextrorphan decreased throughout gestation (Tracy et al., 2005), suggesting an approximately 2-fold increase in CYP2D6 activity. However, urinary ratios depend on changes in renal clearance and alternative pathways such as glucuronidation, and as such, interpretation of this quantitative analysis is challenging. Clonidine oral clearance increases 2-fold during pregnancy with no change in clonidine renal clearance, suggesting that the metabolic clearance presumably mediated by CYP2D6 (Buchanan et al., 2009; Claessens et al., 2010) was increased during pregnancy. Similarly, paroxetine clearance, likely mediated by CYP2D6, increased approximately 2-fold during gestation (Ververs et al., 2009). Taken together, these data unavoidably leave one to conclude that CYP2D6 function is enhanced during pregnancy, but raise questions of the true magnitude of change. For example, is it appropriate to trust the validated probe data, or should the accumulating data for alternative substrates with less well characterized overall clearance mechanisms, or confounding changes in minor clearance pathways, be emphasized to translate changes in CYP2D6 activity into recommendations of clinical dosage adjustment?

At present, it is not clear whether CYP2B6 activity increases during pregnancy. This is a significant gap in our knowledge, as CYP2B6 contributes to the elimination of many drugs commonly administered during pregnancy. The clearance of efavirenz, a CYP2B6 marker, was unaffected by pregnancy (Cressey et al., 2012), but efavirenz is both

TABLE 1

Summary of changes in P450 probe and sensitive marker drug disposition and in disposition of UGT markers at different stages of pregnancy

Target P450	Marker Drug	Effect on Marker Clearance during Gestation			Reference
		First Trimester	Second Trimester	Third Trimester	
CYP1A2	Caffeine, theophylline	↓	↓	↓	(Carter et al., 1986; Tracy et al., 2005)
CYP2B6	Efavirenz			↔↑[a]	(Cressey et al., 2012)
CYP2D6	Metoprolol (dextromethorphan UR)	(↑)	(↑)	↑	(Hogstedt et al., 1985; Tracy et al., 2005)
				(↑)	
CYP2C9	Phenytoin	↔	↑	↑	(Dickinson et al., 1989; Tomson et al., 1994)
CYP3A4	Midazolam			↑	(Hebert et al., 2008)
UGT1A4	Lamotrigine	↔	↑	↑	(Franco et al., 2008; Ohman et al., 2008)
UGT2B7	Zidovudine			↔	(Watts et al., 1991; O'Sullivan et al., 1993)

UGT, UDP glucuronosyltransferase; UR, urinary ratio.
[a] Efavirenz area under the curve was unaffected, but C_{min} was significantly decreased during the third trimester. Efavirenz is an inducer and inactivator of CYP2B6, and this may confound the findings.

an inducer and an inactivator of CYP2B6, and therefore, these data are difficult to interpret mechanistically. In contrast, decreased methadone concentrations and increased clearance of methadone have been reported in pregnant women (Pond et al., 1985; Wolff et al., 2005), suggesting that CYP2B6 (or CYP3A4) activity or renal clearance of methadone is increased during pregnancy (Dickmann and Isoherranen, 2013). Again, additional research is needed to fully understand the time course of CYP2B6 activity during pregnancy.

In addition to the changes in P450 enzyme activity during pregnancy, there is clear evidence that the activity of some but not all UGT enzymes is altered by pregnancy. This is of importance due to the fact that UGT enzymes often contribute not only to the elimination of the parent drug but also to the elimination of pharmacologically active metabolites or metabolites that are used as markers of P450 activity. For example, circulating concentrations of the antiepileptic drug lamotrigine decreased by about 50% during pregnancy (Franco et al., 2008), and the plasma concentration ratio of lamotrigine glucuronide to lamotrigine increased by about 2-fold in the second and third trimesters of pregnancy compared with postpartum (Ohman et al., 2008). Lamotrigine N-glucuronidation is predominantly mediated by UGT1A4 (Green et al., 1995), and hence, these data suggest that UGT1A4 activity is increased during pregnancy, and has important implications for seizure control in pregnant women taking lamotrigine. In contrast, based on zidovudine and morphine pharmacokinetic data, UGT2B7 activity is unaltered during pregnancy (Anderson, 2005).

In addition to maternal hepatic increases in UGT and P450 expression, fetal UGT and P450 activity is also of note. In this issue of *Drug Metabolism and Disposition*, the mRNA expression of UGT2B7, UGT2B15, and UGT2B17 in fetal tissues including fetal liver, lungs, adrenal glands, and kidneys is shown (Ekstrom et al., 2013). Although the mRNA levels were overall lower than those observed in adult human tissues, it is possible that the fetal UGT enzymes do contribute to detoxification of xenobiotics within the fetus. In another article in this issue (Vyhlidal et al., 2013), a correlation between maternal cigarette smoking and induction of fetal hepatic and lung CYP1A1 and fetal lung CYP1B1 was observed. Although the focus of the manuscript is in validating placental cotinine concentrations as a biomarker for fetal exposure to cigarette smoke, the correlation between this biomarker and fetal P450 induction is of interest.

It has been assumed that the renal clearance of drugs is generally increased during pregnancy due to the fact that glomerular filtration rate increases significantly along with renal blood flow during pregnancy (Anderson, 2005; Abduljalil et al., 2012). However, the clearance of amoxicillin, a drug cleared almost exclusively via the renal route, was unaffected by pregnancy (Muller et al., 2008a,b),

whereas the clearance of the related antibiotic ampicillin was increased during pregnancy (Philipson, 1977; Chamberlain et al., 1993). These data suggest that, despite their similar structures, different active secretion and reabsorption mechanisms that change during pregnancy contribute to amoxicillin and ampicillin renal clearance. It appears that changes in secretory drug transporter activity in the kidney contribute to altered drug disposition and response during pregnancy. Table 2 summarizes the existing data on changes in the disposition of transporter marker substrates (from the FDA list of recommended substrates) during pregnancy. As shown, data on changes in drug transporter activity are even sparser than those on drug-metabolizing enzymes. In a pivotal study evaluating changes in P-glycoprotein activity, digoxin renal and secretion clearance were shown to increase during pregnancy (Hebert et al., 2008). Similarly, metformin secretion by organic cation transporters (OCTs) was shown to increase during the second and third trimesters, but not during the first trimester (Eyal et al., 2010). It is possible that the increase in metformin secretion is also due to increased expression of multidrug and toxic compound extrusions in the kidney, as metformin secretion has been shown to be affected by these transporters (Tanihara et al., 2007; Kusuhara et al., 2011). The published metformin data are unique, as they allow an evaluation of changes in tubular secretion throughout gestation.

Although elimination by secretory processes is important for maternal drug disposition, fetal transporters may also play a critical role in protection of the fetus from the deleterious effects of xenobiotics. For example, in this issue of *Drug Metabolism and Disposition*, Sharma et al. (2013) describe the transport of 17α-hydroxyprogesterone caproate in adult human and fetal hepatocytes, and show that 17α-hydroxyprogesterone caproate, a drug used to prevent preterm labor, is a substrate of multiple transporters in fetal and adult human hepatocytes. The report provides a comparison of transporter expression in adult and fetal hepatocytes, demonstrating that MDR3 and sodium-taurocholate cotransporting polypeptide are predominantly expressed in adult hepatocytes, and overall expression levels of all of the transporters measured, except MRP4, were lower in fetal hepatocytes than in adult hepatocytes (Sharma et al., 2013). Incorporation of these data into a PBPK model for the maternal-fetal unit may permit prediction of how changing fetal liver transporter function during pregnancy influences fetal drug exposure.

Use of Animal Models to Study Changes in Drug Disposition during Pregnancy

For many years, investigators have used animal models to characterize fetal safety and drug disposition during pregnancy (Fig. 1). Early on, the nonhuman primate model was considered to be the most appropriate system to study pregnancy-mediated changes in drug

TABLE 2

Summary of changes in transporter probe and sensitive marker drug disposition at different stages of pregnancy

Transporter	Marker Drug	Effect on Clearance during Gestation			Reference
		First Trimester	Second Trimester	Third Trimester	
P-gp	Digoxin			↑	(Hebert et al., 2008)
OATP1B1	Glyburide[a]			↑	(Hebert et al., 2009)
OCT2	Metformin	↔	↑	↔↑[b]	(Hughes et al., 2006; Eyal et al., 2010; de Oliveira Baraldi et al., 2011)
OAT1	Zidovudine, lamivudine			↔	(Moodley et al., 1998)
OAT3	Acyclovir, zidovudine			↔	(Frenkel et al., 1991; Haddad et al., 1993)

OCT, organic cation transporter; OAT, organic anion transporter; P-gp, P-glycoprotein.

[a] While glyburide is listed as an in vivo substrate of OATP1B1, it is cleared by CYP3A4 and CYP2C9 and is a substrate of *BCRP* (breast cancer resistance protein). Hence, it is not possible to determine which enzyme is responsible for the increased clearance of glyburide during pregnancy in vivo.

[b] The secretion clearance of metformin was significantly increased during the third trimester, although oral clearance was not significantly increased.

disposition, and it was used extensively to evaluate both fetal exposure to drugs and teratogens, and the disposition of xenobiotics during pregnancy. However, recent studies have shown that rodents, especially mice, can be a valuable model to investigate changes in drug disposition during pregnancy and mechanisms of P450 regulation by hormones (Zhang et al., 2008), although as pointed out below there are clear limitations. In this issue of *Drug Metabolism and Disposition*, several investigators provide a characterization of how specific drug-metabolizing enzymes and drug transporters are altered in the pregnant mouse model. For example, *Cyp2d* expression and dextromethorphan metabolism were increased in mouse liver, and the observed change was in agreement with the changes in dextromethorphan metabolism during human pregnancy (Topletz et al., 2013). The specific increase in Cyp2d40 and Cyp26a1 mRNA (Topletz et al., 2013) was also in qualitative agreement with the detected increase in these transcripts in the microarray study reported in this issue (Shuster et al., 2013). Some differences in individual Cyp2d mRNA expression were, however, observed between the two studies, highlighting the challenges of quantifying pregnancy-mediated changes in mice. Interestingly, in the microarray study of the changes in the hepatic and renal mRNA expression of genes related to drug disposition, including P450, UGT, and sulfotransferase genes (Shuster et al., 2013), the authors report that increases in UGT mRNAs were not observed, despite the increase in lamotrigine (UGT1A4) metabolic clearance that occurs during human pregnancy. This issue of *Drug Metabolism and Disposition* also shows that the expression of carboxylesterases is decreased during mouse pregnancy (Fortin et al., 2013). This is of interest as oseltamivir, a drug used to prevent influenza and H1N1 virus infection during pregnancy, is converted to its active entity oseltamivir carboxylate by carboxylesterases (primarily CES-1). During pregnancy, oseltamivir exposure and oral clearance were not altered compared with nonpregnant controls, suggesting carboxylesterase activity is unchanged during human pregnancy (Beigi et al., 2011). However, exposure to oseltamivir carboxylate was lower in pregnant than nonpregnant women. This is most likely due to an increase in the clearance of the metabolite, in the absence of a change in parent drug AUC and a parallel oseltamivir clearance pathway. Better validation of the mouse model and understanding of the mechanisms of decreased carboxylesterase mRNA in the mouse will be helpful to determine the mechanisms of oseltamivir clearance changes in humans.

With regard to drug transport in the pregnant mouse model, it was found that both renal Mdr1b (Yacovino et al., 2013) and Mdr1a (Shuster et al., 2013) expression were decreased during gestation, whereas digoxin renal clearance is increased in humans (Hebert et al., 2008). Similarly, renal Oct2 and Oct3 mRNA was reported to be unchanged in mice, and Oct1 mRNA was downregulated at day 7 of gestation and mildly (1.1-fold) upregulated on day 17 of pregnancy (Yacovino et al., 2013), despite the observed significant increase in metformin renal secretion in humans (Eyal et al., 2010). It is possible that the increase in metformin renal clearance during pregnancy is due to increased expression of MATE instead of OCT3, as MATE1 and MATE2 have been suggested to contribute to metformin renal clearance (Tanihara et al., 2007; Kusuhara et al., 2011). However, the mRNA of Mate1 was also consistently decreased in mouse kidney during gestation (Shuster et al., 2013; Yacovino et al., 2013). Of the renal transporters, only Mrp3 mRNA was increased during pregnancy. As such, a better validation of renal clearance changes in the mouse during pregnancy is needed.

Overall, these available data show that any animal model used to study mechanisms of changes in drug disposition during pregnancy should be validated for the enzyme/transporter of interest and shown to replicate the

phenomenon of interest in humans. Although the data presented collectively provide a foundation for the mouse model as a system to evaluate pregnancy-mediated changes in drug disposition, it is clear that we are still far from understanding the detailed mechanisms of how drug-metabolizing and transport activities are altered during pregnancy. An interesting aspect of the mouse data is that, for transporters that are expressed in multiple tissues such as the placenta, maternal kidney, and the liver and fetal tissues, the regulation of these enzymes during pregnancy appears to be tissue-specific. To gain an understanding of tissue-specific regulation, novel tools clearly need to be developed to allow extrapolation of mechanistic findings to specific tissues in vivo.

Mechanistic Studies Using In Vitro Systems to Evaluate Regulation of Drug-Metabolizing Enzymes and Transporters during Pregnancy

Potentially the most challenging aspect of studying drug disposition during pregnancy relates to establishing a clear link between regulatory control of drug-metabolizing enzymes and transporters by endogenous compounds in vitro and the changes in relevant cell exposure to these endogenous compounds during pregnancy. Just because a compound can elicit an increase or decrease in the expression of an enzyme or transporter in vitro does not necessarily mean that altered systemic or local concentrations of that molecule are responsible for changes in tissue drug metabolism and transport gene expression during pregnancy. For example, three studies in this issue of *Drug Metabolism and Disposition* approach the regulation of P450 enzymes by estrogens, progesterone, and their combination (Choi et al., 2013; Dickmann and Isoherranen, 2013; Papageorgiou et al., 2013). Together with past evaluations of regulation of P450 enzymes by estrogens (Choi and Jeong, 2009; Mwinyi et al., 2010; Choi et al., 2011; Koh et al., 2012), these studies clearly show that pregnancy-related hormones regulate P450 mRNA in vitro. However, as highlighted in the articles, during pregnancy maternal hepatocytes are exposed to a combination of hormones and growth factors that may act in a synergistic or antagonistic manner, and dissecting individual mechanisms and regulators will require more in vivo studies during pregnancy as well as more detailed in vitro studies. The potential role of estrogens and progestins in the regulation of P450 expression during pregnancy is supported by the fact that oral contraceptives have similar effects on P450 activity as observed during pregnancy. For example, oral contraceptives had a similar effect on proguanil to cycloguanil oxidation as pregnancy in vivo, suggesting that hormonal regulation of CYP2C19 takes place (McGready et al., 2003). In agreement, downregulation of CYP2C19 by synthetic and natural estrogens has been shown in vitro (Mwinyi et al., 2010). A similar phenomenon is seen with CYP1A2 and its decreased activity during pregnancy.

The regulation of drug transporter genes by estrogens during pregnancy is also poorly understood, but may involve a combination of estrogen receptor activation and activation of constitutive androgen receptor by estrogens (Koh et al., 2012). In this issue of *Drug Metabolism and Disposition*, hepatic MRP3 expression is shown to be induced by synthetic and natural estrogens via estrogen receptors (Ruiz et al., 2013). This suggests that, during pregnancy, MRP3 expression would be increased. However, in the mouse microarray study (Shuster et al., 2013), a decrease in MRP3 mRNA in the liver was observed during pregnancy, whereas in a separate study, kidney MRP3 was increased (Yacovino et al., 2013), suggesting that the effects of pregnancy-related hormones on specific gene regulation are tissue-specific. These data also show that validation studies for mechanistic predictions are critical to fully understand how pregnancy influences

drug disposition. Given the broad spectrum of genes whose expression appears altered during pregnancy, it might be quite informative to compare the profile of gene expression changes in pregnant animals with the location of estrogen and progestin response elements similar to those identified in the recent human ENCODE (The Encyclopedia of DNA Elements) project (Cheng et al., 2012). Perhaps one could define pregnancy-induced changes in drug metabolism and transport processes as part of a larger epigenetic transformation initiated by the marked change in hormone exposure throughout the body.

Finally, even greater challenges are related to the somewhat sparse knowledge about the time course of changes in signaling factors throughout the course of a pregnancy, and how these correlate with the changes in disposition of probe and clinically relevant drugs. This, of course, is critical for accurate predictions of maternal and fetal drug exposure when drug therapy can be required or desired at any stage of a pregnancy. Again, some of the research described in this special issue of *Drug Metabolism and Disposition* begins that characterization of gestational age–dependent change in maternal and fetal drug biotransformation and transporter function, and points the way forward to safer and more efficacious drug treatments in pregnancy whenever they are needed.

Conclusions

In light of the existing clinical, animal, and mechanistic data, it has become quite clear that pregnancy alters drug disposition in a significant manner. Despite a considerable increase in the number of studies published recently on drug disposition during pregnancy, significant gaps in our knowledge still remain. An improvement in our understanding of pregnancy-mediated changes in drug disposition and in the predictability of these changes will depend largely on our ability to integrate data from mechanistic in vitro studies and in vivo animal studies with clinical findings in pregnant women (Fig. 1). The studies published in this special issue of *Drug Metabolism and Disposition* achieve this in some respects, and indicate that we are ready to embark on a mechanistically driven stage of investigation that makes significant use of PBPK modeling. For example, we now have data available to show that CYP2C19 activity is downregulated by estrogens in vitro, and there is a clinical implication that CYP2C19 activity is indeed decreased during some stages of gestation. These data can be used to predict and simulate, using tools such as PBPK modeling, how probe disposition (e.g., omeprazole) would change during pregnancy. Appropriate clinical studies can then be conducted to address the existing gaps in our knowledge. On the other hand, due to clear changes in the pharmacodynamic response to many drugs during pregnancy, such as with oral hypoglycemic agents, a concerted effort may be needed to evaluate the PK-PD relationships of drugs during pregnancy and achieve the desired improvement in clinical outcomes.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Isoherranen, Thummel.

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