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CHANGES IN HEPATIC DRUG METABOLIZING ENZYMES DURING PREGNANCY

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ABSTRACT

Sweeping physiological and biochemical changes occur during pregnancy, and the pharmacokinetics of many drugs are altered over the course of gestation. Small clinical studies suggest that the activity of many of the drug metabolizing enzymes is altered during pregnancy. For example, CYP1A2 activity decreases during pregnancy while CYP2D6 activity increases during pregnancy. As more than half of all women require prescription drugs at some point during pregnancy, it is vital to understand how pregnancy affects the drug metabolizing enzymes. We propose that during pregnancy, circulating sex steroids, vitamins and cytokines act directly on hepatocytes to up- and down-regulate drug metabolizing enzymes through transcriptional mechanisms leading to altered enzyme synthesis. Furthermore, we propose that because pregnancy acts on enzyme expression, pharmacokinetic changes can be extrapolated from one substrate to another to predict pharmacokinetic changes during pregnancy.

KEYWORDS: Hepatic drug metabolizing enzymes, pregnancy.

INTRODUCTION

Almost every aspect of the physiology involved in drug disposition is altered during pregnancy. Physiological changes include alterations to the circulatory system, body volume and composition, and the digestive system.[1] Nausea and acid reflux may result in an aversion to oral medications and problems with compliance. Also, vomiting could interrupt absorption. Two studies have investigated whether gastric emptying decreases during pregnancy using ultrasound or by following acetaminophen absorption and found no differences.[2] Pregnancy has been suggested to slow gut motility and increase gastrointestinal transit time during late pregnancy. Although these physiological changes could theoretically

impact absorption during pregnancy, changes in the rate or extent of absorption have not been reported.[3]

The average pregnant women gain approximately 12.5 kg of mass over the course of her pregnancy. The fetus, placenta and amniotic fluid account for 3.4 kg, 0.65 kg and 0.8 kg of this gain, respectively. There is also a 1.4 L increase in extracellular water in the third trimester. The blood volume increases by 1.45 L during pregnancy, mainly due to an approximately 50% increase in plasma volume. This large increase in body volume, especially at the end of pregnancy, may translate into an increased volume of distribution for some drugs including theophylline[4], ampicillin, and azithromycin. There are also changes in plasma protein binding during pregnancy. Although Chu et al. found that a-1-acid glycoprotein (AGP) was not significantly different during pregnancy, Tsen et al. found that both albumin and AGP concentrations decreased during pregnancy and that this decrease in plasma proteins was associated with an increased free fraction (fu) of bupivacaine.[5]

Decreased protein binding during pregnancy could theoretically lead to increased unbound concentrations of intravenously administered, high clearance drugs during pregnancy, however, this phenomenon has not been investigated during pregnancy. The major concern with decreased protein binding is that it will decrease total drug concentrations independently of changes in unbound drug clearance. This could lead a clinician to increase the dose; however, dose adjustments should be based on unbound drug concentrations, which depend only on clearance. Phenytoin and valproate are classic examples of this, however, the unbound concentrations of most other drugs are rarely if ever measured.[6]

It is not clear whether increased cardiac output during pregnancy also increases blood flow to the liver. Increased liver blood flow would increase the delivery of drugs and therefore the systemic clearance of drugs, particularly drugs with a high extraction ratio. Two studies using the high extraction ratio compounds bromsulfalein and indocyanine green[7] indicated that clearance and therefore liver blood flow in pregnant patients did not differ from non-pregnant patients. However, a more recent study using ultrasound found that liver blood flow increased in the third trimester compared to the second trimester.[8]

BACKGROUND

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.[9]

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, 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.[10]

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.[11] 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 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. 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.[12]

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.[13] 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. 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.[14]

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 and ~50% increase for metformin 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.[15]

Another recently described example of altered drug pharmacokinetics during pregnancy involves the immunosuppressant drug tacrolimus.[16] 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.

Following the thalidomide tragedy of the late 1950s and early 1960s, pregnant women and even women of childbearing age were excluded from clinical drug trials. As a result, today there is inadequate knowledge of drug safety and efficacy in pregnant women. However, women frequently use drugs during pregnancy. Life-threatening conditions, such as infection, hypertension and gestational diabetes, arise during pregnancy. Many women require treatment for chronic conditions such as asthma, epilepsy, HIV, thrombophilia and mental illness during pregnancy.

In addition, nicotine replacement and methadone maintenance therapy are used to ameliorate withdrawal symptoms for women addicted to tobacco and opiates, respectively. Accordingly, 60-93% of women use prescription drugs at some point during pregnancy. Andrade et al. found that 78.6% of the drugs used during pregnancy were classified as either FDA Pregnancy Category B or C, indicating that the majority of drugs used by pregnant women have not been adequately studied in this population.

Changes in Pharmacokinetics

During Pregnancy One way to manage unknown fetal risks during pregnancy is to prescribe the minimum effective dose to the mother. Unfortunately, it may be difficult to determine the appropriate dose as changes in drug metabolism, gut motility, cardiac output, renal blood flow, plasma volume, and plasma protein binding could alter the pharmacokinetics of many drugs during pregnancy. Increased concentrations of drugs with a narrow therapeutic index such as theophylline may lead to toxicity or expose the fetus to higher concentrations than are necessary. Conversely, decreased drug concentrations may lead to a loss of efficacy. For example, low maternal concentrations of anti-HIV drugs like nelfinavir may contribute to drug resistance and mother-to-child-transmission of HIV. Similarly, there are reports that pregnant women need increased doses of antidepressants to maintain euthymia.[17] By employing a mechanistic approach to study pregnancy-mediated changes in pharmacokinetics, we can understand and anticipate changes in disposition and exposure. In order to maximize therapeutic benefit while minimizing toxicity and fetal drug exposure, a thorough understanding of drug disposition during pregnancy is required.

Drug Metabolism During Pregnancy

The cytochrome P450 enzymes are involved in the metabolism of 95% of drugs and of these the most important are CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Changes in activity during pregnancy are isoform-specific; CYP3A4, CYP2C9 and CYP2D6 activity

increases whereas CYP2C19 and CYP1A2 activity decreases. These studies suffer from a number of limitations such as a small number of drugs studied, a small number of subjects per study and a general lack of data from early pregnancy. Nonetheless, these studies have been invaluable in advancing our understanding of drug disposition during pregnancy.[18]

Changes in CYP1A2 activity during pregnancy will be a major focus of this thesis. CYP1A2 is remarkable because, as mentioned previously, CYP1A2 activity appears to decrease during pregnancy. The oral clearance of the classical CYP1A2 probe substrate, theophylline, decreased during pregnancy by 6% and 37% during mid- and late-pregnancy, necessitating dose reductions in some cases. Single time point concentrations of the endogenous CYP1A2 activity marker, melatonin, increased by 80% during late pregnancy.[19]

It is not known whether pregnancy affects the rate of melatonin formation and secretion. More recently, Tracy et al. found that caffeine salivary clearance, which should reflect liver CYP1A2 intrinsic clearance, decreased progressively by 33%, 42% and 65% during the first, second and third trimester, respectively. Together, these studies provide compelling evidence that CYP1A2 activity decreases during pregnancy.[20]

The basis for Changes in Drug Metabolism

The biochemical basis for pregnancy-mediated changes in drug metabolism is not well understood. Alterations in activity may result from changes in gene transcription or protein expression or in the intrinsic properties of the enzyme itself.[21]

We hypothesized that changes in drug metabolism result from changes in hepatic gene transcription and enzyme expression, however, this has not been measured in pregnant women. This is due to the difficulty of obtaining pregnant human liver tissue; it is unethical to conduct medically unnecessary, invasive and potentially harmful biopsies on pregnant women. Generally, liver tissue from pregnant women is only available in the case of accidental death.[22]

Table 1.1: Changes in Cytochrome P450 Enzyme Activity During Pregnancy

ENZYME	SUBSTRATE	OBSERVED CHANGE	REFERENCE
CYP1A2↓	Theophylline	Oral Clearance ↓ 37%	[7]
	Caffeine	Oral Clearance ↓ 65%	[28]
	Endogenous Melatonin	Concentration ↑ 80%	[27]
CYP2A6↑	Nicotine	Non-renal Clearance ↑ 64%	[74]
CYP2B6 ↑	Methadone	Unbound Oral Clearance ↑64%	[75]
CYP2C9 ↑	Phenytoin	Free Concentration ↓ 18%	[76]
CYP2C19↓	Proguanil / cycloguanil	Plasma Metabolic Ratio ↑ 62%	[29]
CYP2D6↑	Dextromethorphan / dextrorphan	Urinary Metabolic Ratio ↓ 48%	[28]
	Dextromethorphan / dextrorphan	Plasma Metabolic Ratio ↓ 53%	[32]
	Metoprolol	Oral Clearance ↑ 5-fold	[31]
	Fluoxetine	Concentration↓ 60%	[77]
	Fluoxetine	Concentration↓ 61%	[78]
CYP3A4 ↑	Midazolam	Unbound Oral Clearance ↑ 171%	[34]
	Dextromethorphan / 3-hydroxymorphinan	Urinary Metabolic Ratio ↓ 35%	[28]
	Cortisol to 6-hydroxycortisol	6-hydroxycortisol A_{urine} ↑ 208%	[35]
	Glyburide	Unbound Oral Clearance ↑ 105%	[36]
	Nelfinavir	Concentration↓ 29%	[9]

Animal Studies

Most animal studies investigating changes in drug disposition during pregnancy have focused on the rat, as it is a classical animal model for reproductive toxicity. As in humans, concentrations of albumin and a-1-acid glycoprotein (AGP) in the rat decrease during pregnancy. In pregnant rats, hepatic portal blood flow increases 38% whereas human data regarding changes to liver blood flow are equivocal. In rats, liver mass increases as much as 50% during late-pregnancy, however, parallel changes have not been reported in pregnant women.[23] Caffeine N-demethylation is a selective marker of CYP1A2 activity in male rats. Following oral administration of caffeine, the AUC of caffeine in pregnant rats was not different from that in non-pregnant rats however the metabolic AUC ratio of theobromine over caffeine decreased by 90%, suggesting reduced CYP1A2 activity in rats during pregnancy. In a study designed to measure the effects of smoking in pregnant rats, CYP1A2 activity increased whereas CYP1A2 protein expression decreased.[24]

The basis for this discrepancy between activity and protein expression is not clear. Furthermore, the results of our study, do not agree and indicate that pregnancy decreases both CYP1A2 activity and expression. In the rat, CYP2C activity and expression did not change during pregnancy[25], whereas in pregnant women the activity of CYP2C9 increases while the activity of CYP2C19 decreases. In pregnant women, CYP2D6 activity is increased, whereas in pregnant rats CYP2D2 activity and mRNA decreased.[26]

Due to its importance in drug metabolism, CYP3A has been studied during pregnancy in the rat, monkey, and mouse. In the rat, hepatic CYP3A9 protein expression was decreased to 1.5% of control values on day 13 of pregnancy[44] whereas in pregnant women CYP3A activity increases during pregnancy. In the macaque, midazolam unbound oral clearance and CYP3A protein expression were not increased during pregnancy. In the mouse, CYP3A activity and protein expression increased during pregnancy.[27]

Table 2: Animal Studies of Cytochrome P450 Enzyme Activity and Expression During Pregnancy.

ENZYME	SUBSTRATE	OBSERVED CHANGE
CYP1A2↓	Theophylline	Oral Clearance ↓ 37%
	Caffeine	Oral Clearance ↓ 65%
	Endogenous Melatonin	Concentration ↑ 80%
CYP2A6↑	Nicotine	Non-renal Clearance ↑ 64%
CYP2B6 ↑	Methadone	Unbound Oral Clearance ↑64%
CYP2C9 ↑	Phenytoin	Free Concentration ↓ 18%
CYP2C19↓	Proguanil / cycloguanil	Plasma Metabolic Ratio ↑ 62%
CYP2D6↑	Dextromethorphan / dextrorphan	Urinary Metabolic Ratio ↓ 48%
	Dextromethorphan / dextrorphan	Plasma Metabolic Ratio ↓ 53%
	Metoprolol	Oral Clearance ↑ 5-fold
	Fluoxetine	Concentration↓ 60%
	Fluoxetine	Concentration↓ 61%
CYP3A4 ↑	Midazolam	Unbound Oral Clearance ↑ 171%
	Dextromethorphan / 3-hydroxymorphinan	Urinary Metabolic Ratio ↓ 35%
	Cortisol to 6-hydroxycortisol	6-hydroxycortisol A_{urine} ↑ 208%
	Glyburide	Unbound Oral Clearance ↑ 105%
	Nelfinavir	Concentration↓ 29%

RESULTS AND DISCUSSION

As discussed previously, pregnancy alters the disposition of many drugs. Down-regulation of CYP1A2 could lead to higher concentrations of CYP1A2 substrates during pregnancy. For example, the oral clearance of theophylline, a CYP1A2 substrate with a narrow therapeutic index, decreased 35% during late pregnancy necessitating dose reductions for some patients to avoid toxicity.[28] More than 60% of pregnant women use drugs other than prenatal vitamins or nutritional supplements so it is vital to understand the mechanisms underlying these pharmacokinetic changes. Prior studies have used the mouse and rat as model animals to study changes in cytochrome P450 activity and expression during pregnancy. In the mouse, Cyp3a activity and protein expression increases during pregnancy and these observations are in accordance with the increased activity of CYP3A observed in pregnant women.[29]

In contrast, rat CYP2C activity and expression did not change during pregnancy, whereas in pregnant women the activity of CYP2C9 increases while the activity of CYP2C19 decreases. In pregnant women, CYP2D6 activity is increased, whereas in pregnant rats CYP2D2 activity and mRNA decreased.[40] CYP1A2 has also been studied in pregnant rats, however, the results of these studies are equivocal. Following oral administration, the AUC of caffeine in pregnant rats was not different than in non-pregnant rats although the AUC of its metabolites (theobromine and theophylline) decreased, suggesting reduced CYP1A2 activity.[30] In another study, methoxyresorufin O-demethylation (MROD) in liver microsomes from pregnant rats decreased while CYP1A2 protein increased. The discrepancy between activity and protein expression was not clear and neither of these studies measured CYP1A2 mRNA during pregnancy. Therefore the primary aim of this study was to investigate whether CYP1A2 activity and expression decrease during rat pregnancy via transcriptional down-regulation.[31]

Caffeine and methoxyresorufin were found to be selective CYP1A2 probe substrates for rat CYP1A2. Rat CYP1A2 is the major P450 isoform catalyzing caffeine N-demethylation to theobromine, paraxanthine, and theophylline although CYP2C6 and CYP2C11 also contributed to the formation of theophylline. CYP2C6 was able to form theobromine and paraxanthine albeit much less efficiently than rat CYP1A2. A recent study focusing on the male rat also found that caffeine 1-N- and 3-N-demethylation were CYP1A2- selective. This study also reported that caffeine 8-hydroxylation is a selective probe reaction for CYP1A2

and that this pathway is more rapid than N-demethylation, however, this reaction was not used due to the unknown contribution of rat CYP3A, which could be altered during pregnancy. CYP1A2 is the major P450 isoform catalyzing MROD with a minor contribution from CYP2C6. Further characterization with the inhibitor a-naphthoflavone suggests that this reaction is selective for CYP1A2. Significant decreases in hepatic CYP1A2 activity were observed during pregnancy. The rate of caffeine N-demethylation to theobromine decreased to 48% and 52% of control in liver microsomes prepared from rats during mid- and late-pregnancy. These changes are similar to those observed in pregnant human women, where caffeine oral clearance decreased 45% and 55% during mid- and late-pregnancy, respectively. Good agreement between caffeine and methoxyresorufin was observed; the rate of MROD decreased to 50% and 36% of control during mid- and late-pregnancy in the rat. A kinetic experiment suggests that pregnancy-mediated changes in MROD are due to changes in Vmax, which decreased 56% and 51% during mid- and late-pregnancy, respectively. These results have important implications, as the rat is a common model species for studying drug metabolism and developmental toxicity. CYP1A2 can metabolize endogenous estrogens such as 17-P-estradiol and estrone.[32]

Therefore, we investigated the possibility that during pregnancy, rising levels of these hormones could inhibit the CYP1A2 activity directly. To test this hypothesis, 17-P-estradiol, estrone, estriol, and progesterone were each co-incubated with methoxyresorufin and 40% maximal inhibition was observed at supra-physiological concentrations (20,000 nM) of these hormones. The median concentrations of 17-P-estradiol and progesterone in women during the third trimester of pregnancy were 24 nM and 232 nM, respectively.[84] Therefore, it is unlikely that circulating hormones inhibit CYP1A2 metabolism directly during pregnancy. As sex steroids did not efficiently inhibit CYP1A2 activity, CYP1A2 expression during pregnancy was measured. CYP1A2 protein expression as measured by western blot decreased to 67% and 71% of control during mid- and late-pregnancy, respectively. This was likely due to decreases in CYP1A2 transcription as CYP1A2 mRNA, as measured by real-time PCR, significantly decreased to 73% of control during late pregnancy. However, the magnitude of change was smaller than that observed for CYP1A2 protein suggesting that post-transcriptional regulation may also play a role.

The Cytochrome P450 enzymes catalyze the metabolism of drugs, dietary constituents, and endogenous hormones. The activities of several of these drug-metabolizing enzymes are

altered during pregnancy. As shown in Chapter 2, CYP1A2 activity decreased in the rat during late pregnancy due to decreased CYP1A2 mRNA and expression. Additionally, although CYP2D6 activity increased in pregnant women, rat CYP2D activity decreased during pregnancy due to decreased CYP2D1, CYP2D2, and CYP2D4 mRNA expression. The molecular mechanisms governing these changes are not known however the sex steroids have been suggested to alter Cytochrome P450 activity during human pregnancy. Concentrations of estradiol and progesterone rise dramatically during human pregnancy.[33]

CYP1A2 activity may be down-regulated by increasing concentrations of estrogen and progesterone; post-menopausal women had lower caffeine metabolic ratios after 8 weeks of estradiol treatment and estradiol-containing hormone replacement therapy significantly increased concentrations of the CYP1A2 probe, tacrine. In contrast to these data, CYP1A2 mRNA in rat hepatocytes was not affected by 17-p-estradiol treatment. There is also evidence that steroids regulate the CYP2C family; women using oral contraceptives had higher omeprazole metabolic ratios and testosterone-induced CYP2C18 and CYP2C19 in transgenic female mice.[34] Testosterone treatment also upregulated CYP2C and down-regulated CYP2D protein expression in female rats.[35] Interestingly, CYP2C12 activity and expression were not different in the rat during pregnancy. Based on these equivocal results, the primary goal of this study was to determine whether 17-P-estradiol, progesterone, and testosterone regulated CYP1A2, CYP2C12, CYP2D1, CYP2D2, and CYP2D4 mRNA expression in the rat during pregnancy.

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