Short Communication

Altered Cytochrome P450 Expression in Mice during Pregnancy

Received August 14, 2010; accepted October 22, 2010

ABSTRACT:

Human pregnancy is known to influence hepatic drug metabolism in a cytochrome (P450)-specific manner. However, the underlying mechanisms remain unknown, in part due to a lack of experimental models to study altered drug metabolism during pregnancy. In this study, we examined how pregnancy influences expression of major P450 isoforms in mice. Liver tissues were isolated from female FVB/N-mice at different gestational time points: pre-pregnancy, 7, 14, and 21 days of pregnancy, and 7 days postpartum. mRNA expression levels of major P450 isoforms (Cyp1a2, Cyp2a5, Cyp2b10, Cyp2c37, Cyp2d22, Cyp2e1, Cyp3a11, and Cyp3a41) in the liver tissues were determined by quantitative real-time poly-

merase chain reaction. Whereas Cyp2a5 expression was unchanged, Cyp3a41 expression was significantly increased during pregnancy. In contrast, expression of Cyp1a2, Cyp2c37, Cyp2d22, Cyp2e1, and Cyp3a11 was decreased. Expression of Cyp2d22 and Cyp2e1 isoforms correlated with that of peroxisome proliferator-activated receptor (PPAR) α in the mouse livers, suggesting potential involvement of PPAR α in down-regulation of the P450 expression during pregnancy. Effects of pregnancy on expression of other P450 mouse isoforms as well as on *in vivo* drug disposition remain to be characterized. These results provide a guide for future studies on P450 regulation during pregnancy.

Introduction

Human pregnancy affects hepatic drug metabolism in a cytochrome P450 (P450) pathway-specific manner. During pregnancy, elimination of drugs metabolized by CYP2A6, CYP3A4, CYP2D6, and CYP2C9 is increased, whereas elimination of CYP1A2 and CYP2C19 substrate drugs is decreased (Dempsey et al., 2002; Anderson, 2005; Hodge and Tracy, 2007). The underlying mechanisms remain unknown, in part due to a lack of experimental models to study altered drug metabolism during pregnancy.

The effects of pregnancy on hepatic drug metabolism have been extensively examined in rats, the commonly used animal model for pharmacological studies (Guarino et al., 1969; Neale and Parke, 1973; Borlakoglu et al., 1993; He et al., 2007). Pregnancy generally decreases P450 contents and activities, as well as mRNA or protein expression of many other drug-metabolizing enzymes, per gram of rat liver. For example, pregnancy reduced activities of ethoxyresorufin-*O*-deethylation and aminopyrine *N*-demethylation (Borlakoglu et al., 1993) as well as aniline hydroxylation and ethylmorphine *N*-demethylation (Guarino et al., 1969). Results from cDNA microarray experiments also revealed down-regulation of CYP2A1, CYP2D2, CYP2C23, and CYP2E1 in livers of pregnant rats (He et al., 2007). In general, these

findings do not correspond to the drug metabolism changes shown in pregnant women (Dempsey et al., 2002; Anderson, 2005; Hodge and Tracy, 2007).

Mice are another commonly used animal model in pharmacology and genetics studies, based on their litter size and available technologies to manipulate their genome. Potential interspecies differences between rats and mice have been reported in regards to regulation of P450 expression, especially pertaining to ligand specificity for transcriptional regulators of P450 (Graham and Lake, 2008); however, the effects of pregnancy on mouse P450 expression have not been studied extensively. A recent study has demonstrated that pregnancy increases mRNA expression of Cyp3a16, Cyp3a41, and Cyp3a44 in mice (Zhang et al., 2008). The increased Cyp3a expression was, in part, found to be responsible for enhanced metabolism of glyburide in pregnant mice (Zhou et al., 2010), providing a mechanistic basis for the increased elimination of glyburide in pregnant women. This result suggests that mice may serve as a potential animal model to study changes in P450-mediated drug metabolism during pregnancy. The effects of pregnancy on expression of other mouse P450 isoforms remain unknown.

Expression of P450 enzymes are modulated by actions of nuclear receptors or immunomodulators. Cytokines released during systemic inflammation, such as interleukin (IL)-6, IL-1 β , tumor necrosis factor (TNF) α , and interferon (IFN) γ , are known to down-regulate hepatic expression of major P450 isoforms (Aitken et al., 2006). On the other hand, hepatic transcription factors, such as pregnane X receptor (PXR) or constitutive androstane receptor (CAR), play critical roles in up-regulation of hepatic P450 expression. Results from a previous study have shown decreased CAR expression during mouse pregnancy (Zhang et al., 2008). The second trimester of human pregnancy

This work was supported in part by the National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health and Human Development [Grant HD055313]; the National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health and Human Development [Grant K12-HD055892] (fellowship to H.J.); and the National Institutes of Health National Center for Research Resources [Grant UL1-RR029879] (to H.X.).

Article, publication date, and citation information can be found at <http://dmd.aspetjournals.org>.

doi:10.1124/dmd.110.035790.

ABBREVIATIONS: P450, cytochrome P450; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; PXR, pregnane X receptor; CAR, constitutive androstane receptor; qRT-PCR, quantitative real-time polymerase chain reaction; C_T, cycle threshold; ANOVA, analysis of variance; PPAR, peroxisome proliferator-activated receptor; AhR, Aryl hydrocarbon receptor; ER, estrogen receptor; WY-14,643, 4-chloro-6-(2,3-xylyldino)-2-pyrimidinylthioacetic acid.

is characterized by an inflammatory environment manifested by T-helper type 2 cell cytokines, such as IL-6 (Creasy et al., 2009), although the inflammatory environment of mouse pregnancy is yet to be defined. Involvement of these P450 regulators in altered drug metabolism during pregnancy remains unknown.

The aim of this study was to examine how pregnancy influences expression of major P450 isoforms in mice to establish mice as a potential animal model to study altered drug metabolism during pregnancy. We also explored potential mechanisms underlying altered P450 expression during mouse pregnancy by examining how pregnancy influences expression of major transcriptional regulators for P450 expression.

Materials and Methods

Animals. All pregnant and virgin FVB/N-mice were housed under controlled temperature ($20 \pm 2^\circ\text{C}$), relative humidity (50–60%), and controlled lighting (lights on 6:00 AM–6:00 PM) conditions, and food and water were provided ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University at Buffalo. Adult female (8 weeks old) mice were mated with male mice of same age. The 2nd day after mating was assumed as gestational day 1 for those female mice demonstrating sperm plug. At gestational day 7, 14, 21, and 7 days after delivery, the female mice were sacrificed and liver tissues were collected. Age-matched virgin female mice were used as controls (gestational day 0). All tissues were gently washed in 4°C saline (kept on ice) and then stored at -80°C before use.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction. Approximately one third of a whole liver tissue from each mouse was pulverized in liquid nitrogen, and total RNAs were isolated using TRIzol (Invitrogen, Carlsbad, CA). The quality of total RNA was determined by measuring the A_{260}/A_{280} ratio (greater than 1.8), and RNA integrity was further confirmed on agarose gels. One microgram of total RNA was used as template for cDNA synthesis using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). With the cDNA as template, quantitative real-time polymerase chain reaction (qRT-PCR) was performed using StepOnePlus Real-Time PCR System and TaqMan Gene expression assays (Applied Biosystems). TaqMan probes for mouse Cyp1a2 (Mm00487224_m1), Cyp2a4/Cyp2a5 (Mm00487248_g1), Cyp2b10 (Mm00456588_mH), Cyp2c37 (Mm00833845_m1), Cyp2d22 (Mm00530542_m1), Cyp2e1 (Mm00491127_m1), Cyp3a11 (Mm00731567_m1), Cyp3a41 (Mm00776855_mH), and β -actin (Mm00607939_s1) were purchased from Applied Biosystems. For immune modulators and transcription factors, we used a SYBR Green RT-PCR Reagents Kit (Applied Biosystems). Primers used for the detection are shown in Table 1. The fold change in mRNA levels during pregnancy was determined after normalizing the gene expression levels by those of β -actin ($2^{-\Delta\Delta C_t}$ method) (Schmittgen and Livak, 2008). All qRT-PCR experiments were performed in duplicate for each gene, and the results were verified by using at least two different sets of cDNA made from the same RNA.

Statistical Analysis. All data were analyzed using SAS (version 9.1; SAS Institute, Cary, NC). One-way analysis of variance (ANOVA) was performed,

followed by an unpaired Student's *t* test for comparison with the prepregnancy level. Correlation between mRNA expression of P450 isoforms and transcription factors (or inflammatory markers) was determined by the Spearman rank analysis and expressed as the corresponding correlation coefficient (*r*). Post hoc Bonferroni correction was performed to adjust for the Type I error rate ($= 0.05$) for 20 ANOVA and 96 correlation tests. Our study had 80% power to detect a change greater than 82% from the prepregnancy values at the 5% significance level (PASS software, Kaysville, Utah).

Results

Isoform-Specific Effects of Pregnancy on P450 Expression.

Mouse P450 enzymes in drug-metabolizing Cyp1, 2, and 3 families amount to over 30 different isoforms compared to 20 isoforms in humans (Martignoni et al., 2006). We examined mRNA expression of eight major P450 isoforms (Cyp1a2, Cyp2a5, Cyp2b10, Cyp2c37, Cyp2d22, Cyp2e1, Cyp3a11, and Cyp3a41) in livers of virgin, pregnant (7, 14, and 21 days of pregnancy; P7, P14, and P21, respectively), and 7-day postpartum (PP7) mice. Their expression was normalized by that of β -actin, which was not influenced by pregnancy ($p = 0.08$, based on comparison of C_T values). Results from ANOVA testing showed that significant differences existed among different gestational stages for all P450 isoforms except for Cyp2a5: Cyp1a2 ($p = 0.0002$), Cyp2b10 ($p = 0.0007$), Cyp2c37 ($p = 0.0003$), Cyp2d22 ($p < 0.0001$), Cyp2e1 ($p = 0.0001$), Cyp3a11 ($p = 0.0024$), and Cyp3a41 ($p = 0.0001$). Pregnancy decreased expression of five P450 isoforms compared to prepregnancy levels: Cyp1a2, Cyp2c37, Cyp2d22, Cyp2e1, and Cyp3a11 (Fig. 1). Expression of Cyp2d22 and Cyp2e1 was decreased as early as P7 and had not recovered by PP7. In contrast, expression of Cyp2c37 and Cyp3a11, after reaching a nadir at P14 or P21, showed a trend toward recovery to the prepregnancy level at PP7. Expression of Cyp3a41 was up-regulated in mouse pregnancy, a result consistent with a previous report (Zhang et al., 2008). Cyp2b10 expression was increased after delivery. Taken together, these results indicate that, although pregnancy generally decreases expression of major P450 isoforms in mice, the effect of pregnancy and delivery on P450 expression is apparently isoform-specific.

Changes in Expression of Inflammatory Markers and Hepatic Transcription Factors. To explore the potential mechanisms underlying pregnancy-mediated changes in P450 expression, we examined the expression levels of inflammatory markers (IL-2, IL-4, IL-6, IL-10, IL-1 β , IFN γ , and TNF α) and hepatic transcription factors [PXR, CAR, peroxisome proliferator-activated receptor (PPAR) α , Aryl hydrocarbon receptor (AhR), and estrogen receptor (ER) α] in mouse livers at different gestational stages. Results from ANOVA testing showed that significant differences existed among different

TABLE 1
Sequences of primers used in qRT-PCR for detection of immunomodulators and transcription factors

Gene	Sequence	Gene	Sequence
<i>IL-1β</i>	Forward: 5'-GCAACTGTTCTGAACCTCAACT-3' Reverse: 5'-ATCTTTTGGGGTCCGTCAACT-3'	<i>PXR</i>	Forward: 5'-GATGGAGGTCTTCAAATCTGCC-3' Reverse: 5'-GGCCCTTCTGAAAAACCCCT-3'
<i>IL-2</i>	Forward: 5'-GTGCTCCTTGTCACACGCG-3' Reverse: 5'-GGGGAGTTTCAGGTTCTGTA-3'	<i>CAR</i>	Forward: 5'-CCCTGACAGACCCGGAGTTA-3' Reverse: 5'-GCCGAGACTGTTGTTCCATAAT-3'
<i>IL-4</i>	Forward: 5'-GGTCTCAACCCCCAGCTAGT-3' Reverse: 5'-GCCGATGATCTCTCTCAAGTGAT-3'	<i>PPARα</i>	Forward: 5'-AGAGCCCCATCTGTCTCTCTC-3' Reverse: 5'-ACTGGTAGTCTGCAAAACCAAA-3'
<i>IL-6</i>	Forward: 5'-TAGTCCTTCTACCCCAATTTC-3' Reverse: 5'-TTGGTCTCTTAGCCATCTCTTC-3'	<i>ERα</i>	Forward: 5'-CCTCCCGCCTTCTACAGGT-3' Reverse: 5'-CACACGGCACAGTAGCGAG-3'
<i>IL-10</i>	Forward: 5'-GCTCTTACTGACTGGCATGAG-3' Reverse: 5'-CGCAGCTCTAGGAGCATGTG-3'	<i>AhR</i>	Forward: 5'-AGCCGGTGCAGAAAACAGTAA-3' Reverse: 5'-AGGCGGTCTAACTCTGTGTTTC-3'
<i>IFNγ</i>	Forward: 5'-ATGAACGCTACACATGCATC-3' Reverse: 5'-CCATCCTTTTGGCAGTTCTC-3'	<i>β-actin</i>	Forward: 5'-GGCTGTATTCCCTCCATCG-3' Reverse: 5'-CCAGTTGGTAACAATGCCATGT-3'
<i>TNFα</i>	Forward: 5'-CCCTCAGACTCAGATCATCTTCT-3' Reverse: 5'-GCTACGACGTGGGCTACAG-3'

FIG. 1. Effects of pregnancy on mRNA expression of major P450 enzymes. mRNA expression levels of mouse P450 isoforms (Cyp1a2, Cyp2a5, Cyp2b10, Cyp2c37, Cyp2d22, Cyp2e1, Cyp3a11 and Cyp3a41) were determined by qRT-PCR in mouse livers at different gestational time points ($n = 4/\text{group}$; mean $\pm$ S.D.). Data shown are expression levels of each P450 isoform relative to the expression in mouse 1 of the virgin group (arbitrarily set as 1). *, $p < 0.05$ and **, $p < 0.01$ compared with the virgin group.

gestational stages for PPAR α ($p < 0.0001$) (Fig. 2); pregnancy had insignificant effects on expression levels of inflammatory markers or other transcription factors (data not shown).

Next, we examined whether the expression levels of inflammatory markers or transcription factors correlated with those of P450 isoforms in mouse livers at different gestational stages ($n = 20$). Data obtained from Spearman rank analysis are summarized in Table 2. This preliminary examination (including 96 sets of correlation) revealed that expression of Cyp2d22 and Cyp2e1 is correlated with that of PPAR α , suggesting that PPAR α may be involved in altered P450 expression during mouse pregnancy.

Discussion

Our results indicate that pregnancy generally represses expression of major P450 isoforms in mice. Among eight P450 isoforms exam-

ined in this study, only Cyp3a41 showed an increased expression during pregnancy (Fig. 1). Expression of the rest of P450 isoforms was either decreased (Cyp1a2, Cyp2c37, Cyp2d22, Cyp2e1, and Cyp3a11) or not affected (Cyp2a5) by pregnancy. Although it remains to be determined how these changes in mRNA expression lead to altered pharmacokinetics of drugs in mice, the mRNA results suggest that changes in drug metabolism during mouse pregnancy may differ from the clinically observed changes in humans.

The apparent discrepancy in directional changes in P450-mediated drug metabolism between mouse and human pregnancy may be explained by a lack of clear orthologs between the two species, especially for CYP2Cs, CYP2Ds, and CYP3As (Nelson et al., 2004). For example, compared with humans, who have only one isoform in the CYP2D subfamily (i.e., CYP2D6), the mouse has at least nine different isoforms in the Cyp2d subfamily (Martignoni et al., 2006). This attribute is due to significant expansion of P450 gene families in mice (relative to humans) since the divergence of human and rodent lineages ~ 75 million years ago (Mouse Genome Sequencing Consortium, 2002). Considering the large number of P450 isoforms in mice, it appears plausible that P450 isoforms in the same subfamily may respond to pregnancy differently, some of which may better reflect the changes in drug metabolism during human pregnancy than the other P450 isoforms. In fact, pregnancy up-regulates Cyp3a41 expression while it down-regulates Cyp3a11 in mice (Fig. 1). Because the net result is increased protein expression of Cyp3a (Zhang et al., 2008), mice may serve as an animal model to study enhanced CYP3A4-mediated drug metabolism during human pregnancy (Zhou et al., 2010). Further studies are needed to examine how expression of other mouse P450 isoforms are influenced during pregnancy, to establish mice as the animal model to investigate altered drug metabolism during pregnancy. Alternatively, transgenic mice that harbor upstream regulatory regions of human

FIG. 2. Effects of pregnancy on mRNA expression of PPAR α . mRNA expression level of PPAR α was determined by qRT-PCR in mouse livers at different gestational time points ($n = 4/\text{group}$; mean $\pm$ S.D.). Data shown are relative expression of PPAR α compared with mouse 1 of the virgin group (arbitrarily set as 1). *, $p < 0.05$ and **, $p < 0.01$ compared with the virgin group (Student's t test).

TABLE 2

Correlation of mRNA expression levels between immunomodulators/transcription factors and P450 isoforms (n = 20)

Correlation between mRNA expression of P450 isoforms and transcription factors (or inflammatory markers) was determined by the Spearman rank analysis expressed as the corresponding correlation coefficient (*r*).

	Cyp1a2	Cyp2a5	Cyp2b10	Cyp2c37	Cyp2d22	Cyp2e1	Cyp3a11	Cyp3a41
Immunomodulator								
IL-1 β								
<i>r</i>	0.048	0.077	-0.220	0.143	0.178	0.147	0.071	0.068
<i>p</i> value	0.842	0.746	0.351	0.548	0.453	0.537	0.766	0.776
IL-2								
<i>r</i>	0.149	0.039	-0.052	0.013	0.025	-0.032	-0.049	0.167
<i>p</i> value	0.544	0.875	0.834	0.958	0.918	0.898	0.841	0.494
IL-4								
<i>r</i>	0.389	0.214	0.037	0.448	0.578	0.539	0.516	-0.082
<i>p</i> value	0.090	0.365	0.878	0.048	0.008	0.014	0.020	0.731
IL-6								
<i>r</i>	-0.158	0.095	-0.547	-0.076	0.048	0.191	-0.103	0.383
<i>p</i> value	0.506	0.692	0.013	0.749	0.842	0.420	0.665	0.095
IL-10								
<i>r</i>	-0.289	-0.115	-0.539	-0.218	-0.104	0.014	-0.325	0.422
<i>p</i> value	0.216	0.629	0.014	0.355	0.662	0.955	0.162	0.064
IFN- γ								
<i>r</i>	-0.040	-0.018	-0.310	-0.034	0.091	0.113	-0.187	0.074
<i>p</i> value	0.868	0.940	0.183	0.886	0.703	0.634	0.430	0.758
TNF α								
<i>r</i>	-0.312	0.078	-0.535	-0.011	0.019	0.200	-0.166	0.213
<i>p</i> value	0.181	0.744	0.015	0.962	0.935	0.399	0.485	0.366
Transcription factor								
PXR								
<i>r</i>	0.370	0.587	0.278	0.382	0.333	0.361	0.551	-0.026
<i>p</i> value	0.109	0.007	0.235	0.097	0.152	0.118	0.012	0.913
CAR								
<i>r</i>	0.385	0.171	-0.115	0.214	0.472	0.477	0.358	-0.121
<i>p</i> value	0.094	0.471	0.628	0.365	0.036	0.033	0.121	0.611
PPAR α								
<i>r</i>	0.475	0.457	-0.421	0.499	0.773	0.827	0.494	0.063
<i>p</i> value	0.034	0.043	0.065	0.025	<0.0001*	<0.0001*	0.027	0.793
ER α								
<i>r</i>	-0.060	0.097	-0.170	0.544	0.466	0.624	0.589	-0.214
<i>p</i> value	0.803	0.686	0.473	0.013	0.038	0.003	0.006	0.365
AhR								
<i>r</i>	0.521	0.355	0.268	0.256	0.332	0.226	0.210	0.292
<i>p</i> value	0.019	0.125	0.254	0.277	0.153	0.339	0.374	0.212

* $p < 0.0005$ after Bonferroni correction.

P450 genes (Corchero et al., 2001) may present a useful animal model in that interspecies differences in gene regulatory sequences can be overcome.

The underlying mechanisms for the global down-regulation of major P450 isoforms during mouse pregnancy remain unknown. Pregnancy-related physiological changes are likely responsible, which include 1) rising levels of pregnancy hormones, 2) potentially heightened inflammatory response, and 3) possible changes in activity and/or expression of key transcription factors involved in regulation of P450 expression. Pregnancy is accompanied by increasing plasma concentrations of female hormones (estrogens and progesterone), cortisol, and placental growth hormones (Barkley et al., 1979; Masuyama et al., 2001), which may potentially modulate hepatic P450 expression. For example, growth hormone (released in a continuous pattern) increases expression of Cyp3a41 (Sakuma et al., 2002), and estradiol and glucocorticoid can potentiate the inducing effects of growth hormones on Cyp3a41 expression (Sakuma et al., 2004). As suggested previously (Zhang et al., 2008), up-regulation of Cyp3a41 may be attributed to the combined effects of pregnancy hormones on P450 expression. On the other hand, estrogen down-regulates human CYP2C19 expression in an ER α -dependent manner (Mwinyi et al., 2010). These regulatory effects of hormones on P450 expression support potentially significant roles of pregnancy hormones in the reduced P450 expression during mouse pregnancy.

Our results indicate that mouse pregnancy has insignificant effects on hepatic expression of immunomodulators but down-regulates expression of PPAR α (Fig. 2). The lack of effects on other transcription factors or immunomodulators may be due to the small sample size used in this exploratory study. The decreased PPAR α expression strongly correlated with the reduced expression levels of Cyp2d22 and Cyp2e1 (Table 2), suggesting that PPAR α potentially participates in regulation of P450 expression during mouse pregnancy. In fact, it has been shown that a peroxisome proliferator activator (such as WY-14,643) down-regulates CYP2C11 and CYP2C12 while up-regulating CYP4A in rats (Corton et al., 1998; Graham and Lake, 2008) and mice (Savas et al., 2009). However, it is uncertain whether these results can be extrapolated to humans. Significant interspecies difference between rodents and humans has been reported in the PPAR α target genes and subsequent physiological outcomes of PPAR α activation (Graham and Lake, 2008). The physiological roles of reduced PPAR α expression in mouse pregnancy, as well as clinical implications of such changes, remain to be determined.

Taken together, we have shown in this study that pregnancy down-regulates the expression of major P450 isoforms in mice. The information obtained from the current study should be of great value in better understanding the mouse as an animal model and in guiding future studies for identification of an appropriate model system and approaches to investigate P450 regulation during pregnancy.

Acknowledgments

We thank Liam Fischer and Dr. Hye Jin Chung for reading of this manuscript.

Authorship Contributions

Participated in research design: Koh, Yu, and Jeong.

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