Short Communication

Pregnancy Decreases Rat CYP1A2 Activity and Expression

Received August 5, 2010; accepted October 4, 2010

ABSTRACT:

Pregnancy results in increased CYP3A- and CYP2D6-mediated clearance but decreases the clearance of CYP1A2 probe drugs. The aim of this study was to determine whether the decreased CYP1A2 activity during human pregnancy could be explained by decreased expression of CYP1A2 protein and mRNA using the rat as a model. Potential mechanisms leading to decreased CYP1A2 activity and expression were also investigated. Hepatic CYP1A2 activity, protein, and mRNA were measured during mid- and late gestation and compared to nonpregnant control levels. In addition, the effect of 17-β-estradiol and progesterone on CYP1A2 mRNA levels was assessed using rat hepatocytes, and the effect of estrogens or progesterone on CYP1A2 activity in vitro was tested. CYP1A2-mediated probe clearance decreased between 48 and 62% (p < 0.05) during pregnancy, with no difference in CYP1A2 activity between mid- and late pregnancy. This decrease in probe clearance was accompanied by a 33 ± 8% (midpregnancy) and 29 ± 27% (late pregnancy) decrease in CYP1A2 protein expression (p < 0.05) and a 53% decline in methoxyresorufin O-demethylation Vmax (p < 0.05). CYP1A2 mRNA was not significantly different from controls at midpregnancy and decreased by 27 ± 20% (p < 0.05) of control during late pregnancy. Estradiol and progesterone had no effect on CYP1A2 mRNA in rat hepatocytes and did not inhibit CYP1A2 activity. These data demonstrate that pregnancy decreases CYP1A2 activity and expression with a modest effect on CYP1A2 mRNA and suggest that the rat can be used as a model to study mechanisms by which pregnancy decreases CYP1A2 activity in humans.

Introduction

Pregnancy alters the disposition of drugs, and efforts have been made to better understand the enzyme-specific changes as well as the mechanisms that cause these changes. Based on probe substrate studies, the activity of CYP3A4, CYP2C9, and CYP2D6 is increased up to 5-fold during pregnancy but CYP1A2 activity is decreased (Tracy et al., 2005; Anderson, 2005). During late pregnancy, the oral clearance of theophylline decreased by 37% (Carter et al., 1986) and concentrations of melatonin increased by 80% (Kivela, 1991). In a similar manner, caffeine salivary clearance decreased by 33, 42, and 65% during the first, second, and third trimesters of pregnancy, respectively (Tracy et al., 2005). These studies suggest that the mechanisms by which pregnancy alters CYP1A2 activity differ from those leading to increased CYP3A4, CYP2C9, and CYP2D6 activity.

Some of the changes in cytochrome P450 (P450) activity observed in humans have been replicated in animal models, whereas others have shown discrepant results. In the mouse, Cyp3a activity and expression were also investigated. Hepatic CYP1A2 activity, protein, and mRNA were measured during mid- and late gestation and compared to nonpregnant control levels. In addition, the effect of 17-β-estradiol and progesterone on CYP1A2 mRNA levels was assessed using rat hepatocytes, and the effect of estrogens or progesterone on CYP1A2 activity in vitro was tested. CYP1A2-mediated probe clearance decreased between 48 and 62% (p < 0.05) during pregnancy, with no difference in CYP1A2 activity between mid- and late pregnancy. This decrease in probe clearance was accompanied by a 33 ± 8% (midpregnancy) and 29 ± 27% (late pregnancy) decrease in CYP1A2 protein expression (p < 0.05) and a 53% decline in methoxyresorufin O-demethylation Vmax (p < 0.05). CYP1A2 mRNA was not significantly different from controls at midpregnancy and decreased by 27 ± 20% (p < 0.05) of control during late pregnancy. Estradiol and progesterone had no effect on CYP1A2 mRNA in rat hepatocytes and did not inhibit CYP1A2 activity. These data demonstrate that pregnancy decreases CYP1A2 activity and expression with a modest effect on CYP1A2 mRNA and suggest that the rat can be used as a model to study mechanisms by which pregnancy decreases CYP1A2 activity in humans.

Materials and Methods

Chemicals. Potassium phosphate, glucose 6-phosphate, glucose 6-phosphate-dehydrogenase, NADPH, NADP+, magnesium chloride, caffeine, paraxanthine, theobromine, and theophylline were obtained from Sigma-Aldrich (St. Louis, MO). Caffeine was purified from contaminating metabolites using an Agilent 1100 high-pressure liquid chromatography with UV detection system (Agilent Technologies, Santa Clara, CA) equipped with an Econosphere C18 column (250 × 10 mm, 10 μm; Alltech Associates, Deerfield, IL) using an isocratic elution with 12% ammonium acetate (5 mM) and 88% methanol at 3 ml/min. Deuterium-labeled paraxanthine was prepared by reacting D5-methyl iodide with 7-methylxanthine under basic conditions and purified by high-pressure liquid chromatography as described above. D7-theobromine and D7-theobromine were gifts from Tom Kalhorn and Dr. Kent Kunze (University of Washington, Seattle, WA).

Animal Studies and Preparation of Tissue Samples. The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Washington, and the animals were housed with free access to...
food and water under 12-h light/dark cycles. Female Sprague-Dawley rats were mated at 8 weeks of age, and pregnancy was determined by detection of a vaginal plug. Pregnant rats were sacrificed at midpregnancy at day 9 (n = 6) and late pregnancy at day 19 (n = 10). Unmated, age-matched, female rats (n = 6) were used as controls. Livers were harvested, weighed, rinsed in phosphate-buffered saline and stored at −80°C. Microsomes were prepared from individual rat livers according to previously published methods (Rettie et al., 1989). Total microsomal protein concentrations were measured using a Pierce BCA protein assay (Pierce, Rockford, IL), according to the manufacturer’s instructions, using bovine serum albumin as a standard.

**Analysis of CYP1A2 Activity using Caffeine as a Probe.** Caffeine N-demethylation to paraxanthine, theobromine, and theophylline was determined using rat Supersomes (CYP1A2, CYP2B1, CYP2C6, CYP2C11, CYP2C12, CYP2D1, CYP2D2, and CYP3A2; BD Gentest, Woburn, MA). Caffeine (500 μM) was incubated with each isoform (20 pmol) for 30 min with 1 mM NADPH in 100 mM potassium phosphate (pH 7.4). For CYP1A2, the Michaelis-Menten parameters (K_m and V_max) were determined after incubating 50 to 3000 μM caffeine with CYP1A2 (5 pmol) for 15 min. Caffeine N-demethylation was measured as CYP1A2 probe in individual rat livers at a sub-K_m concentration of caffeine (100 μM) with 0.2 mg/ml microsomal protein for 30 min. All incubations were conducted in the linear range for microsomal protein and incubation time.

Paraxanthine, theophylline, and theobromine were extracted using methylene chloride, separated using a SphereClone phenyl column (150 × 4.6 mm; 3 μm; Phenomenex, Torrance, CA) on a Waters 2690 Separations Module (Waters, Milford, MA) with a mobile phase of 18% 10 mM ammonium acetate (pH 7) and 82% methanol at 0.25 ml/min and detected using a Micromass Platform LCZ mass spectrometer (Waters) with positive-mode, selected-ion-monitoring of m/z 183.9 for paraxanthine, theophylline, and theobromine and m/z 180.9 for paraxanthine, theophylline, and theobromine. Peak area ratios were compared to a standard curve (0.2–50 ng/ml) of known concentrations. Intra- and inter-day variability were <16% for theobromine, paraxanthine, and theophylline, and the limit of quantification was 0.2 ng/ml.

**Analysis of CYP1A2 Activity using Methoxyresorufin as a Probe.** MROD was measured in rat Supersome panel (5 pmol/ml for CYP1A2 and 100 pmol/ml for all other isoforms) and in individual rat livers (0.04 mg/ml microsomal protein) using an NADPH-regenerating system (100 mM potassium phosphate buffer, 4 units/ml glucose 6-phosphate-dehydrogenase, 0.13 mM NADP⁺, 3.3 mM glucose 6-phosphate, 3.3 mM magnesium chloride, 200-μl total reaction volume). For the Supersome panel, methoxyresorufin was incubated at 300 nM, and for rat liver microsomes (RLMs) methoxyresorufin was incubated at 25 nM in potassium phosphate buffer. Resorufin formation was monitored at 580 nm (paraxanthine), and MROD were confirmed to be selective markers of CYP1A2 activity in rat liver after incubating caffeine and methoxyresorufin with a panel of rat Supersomes (data not shown). The specificity of MROD as a CYP1A2 probe was confirmed in pooled RLMs. At 200 nM, α-naphthoflavone inhibited 85% of MROD, and 230 ± 27 μM (paraxanthine), and 0.6 ± 0.1 pmol/min·mg (theophylline). MROD appeared more specific for caffeine as CYP1A2 in the Supersome panel (data not shown). The specificity of MROD as a CYP1A2 probe was confirmed in pooled RLMs. At 200 nM, α-naphthoflavone inhibited 85% of MROD, demonstrating that MROD is selective for CYP1A2.

**Results and Discussion**

**Probe Validation.** Caffeine metabolism to theobromine and paraxanthine and MROD were confirmed to be selective markers of CYP1A2 activity in rat liver after incubating caffeine and methoxyresorufin with a panel of rat Supersomes (data not shown). Caffeine metabolites, theobromine instead of paraxanthine was the major metabolite observed in CYP1A2 incubations. Formation of theophylline was less selective for CYP1A2 because CYP2C6 and CYP2C11 metabolized caffeine to paraxanthine. CYP2C6 and CYP2C11, a male-specific isoform, also metabolized caffeine to theophylline. CYP2C6 and CYP2C11 also had a minor activity toward the other N-demethylation reactions. The V_max and K_m estimates for caffeine metabolism by CYP1A2 were 1.7 ± 0.1 pmol/min·mg and 295 ± 42 μM (theobromine), 1.0 ± 0.1 pmol/min·mg and 230 ± 27 μM (paraxanthine), and 0.6 ± 0.1 pmol/min·mg and 323 ± 49 μM (theophylline). MROD appeared more specific for caffeine as CYP1A2 in the Supersome panel (data not shown).

**Pregnancy Mediated Changes in CYP1A2 Activity, Expression, and mRNA during Rat Pregnancy.** CYP1A2-mediated metabolism was decreased by approximately 50% during rat pregnancy regardless of the stage of pregnancy (Fig. 1A). Theobromine formation clearance decreased from 9.2 ± 2.9 (control) to 4.4 ± 1.3 nl/min·mg (p < 0.01) during midpregnancy and to 4.8 ± 2.0 nl/min·mg (p < 0.01) during late pregnancy. Paraxanthine formation clearance decreased from 5.4 ± 1.4 to 2.7 ± 0.9 nl/min·mg (p < 0.01) during midpregnancy and to
2.9 ± 1.2 nl/min (mg) (p < 0.01) during late pregnancy. Theophylline formation decreased from 7.4 ± 1.2 to 3.3 ± 1.4 nl/min (mg) (p < 0.001) during midpregnancy and to 4.6 ± 1.8 nl/min (mg) (p < 0.01) during late pregnancy. In agreement with the results obtained with caffeine as a probe, methoxyresorufin clearance decreased from 220 ± 77 (control) to 119 ± 52 µl/min (mg) (p < 0.05) during midpregnancy and to 133 ± 53 µl/min (mg) (p < 0.05) during late pregnancy.

The V_max of MROD in pooled RLMs decreased significantly (p < 0.0001) from 46 ± 9.1 (control) to 21 ± 3.8 pmol/min (mg) (midpregnancy) and 22 ± 3.8 pmol/min (mg) (late pregnancy), suggesting decreased expression of CYP1A2 or presence of a non-competitive inhibitor in the liver microsomes (Fig. 1B). The K_m for MROD was significantly lower in late pregnancy than controls, suggesting possible modification of CYP1A2 protein itself. In agreement with the decreased V_max of MROD, the relative expression of rat CYP1A2 protein significantly decreased (analysis of variance; p < 0.05) during mid- and late pregnancy to 67 ± 7.8% (p < 0.05) and 71 ± 27% (p < 0.05), respectively, of control rats (Fig. 1C). Despite the significant decrease in CYP1A2 protein expression, no change in CYP1A2 mRNA was observed in midpregnancy, and CYP1A2 mRNA decreased during late pregnancy to 73 ± 20% (p < 0.05) of unmated controls (Fig. 1D). Note that the greatest effect was observed in CYP1A2 activity, followed by a smaller change in CYP1A2 protein expression, and finally the least effect on CYP1A2 mRNA. This result is of interest because with most other P450 enzymes, the largest magnitude of change is observed in mRNA. It is possible that the decreased CYP1A2 protein expression during pregnancy is due to posttranslational regulation rather than effects on CYP1A2 mRNA. However, during late pregnancy, the magnitude of decrease in CYP1A2 mRNA was similar to the protein, suggesting a contribution of transcriptional regulation as well. Based on these results, it is likely that multiple mechanisms contribute to the decreased CYP1A2 activity during pregnancy. This result is noteworthy because the decreased CYP1A2 activity observed in the rat is in close agreement with the 45 and 65% decrease in caffeine oral clearance during mid- and late pregnancy (Tracy et al., 2005). In contrast to the gestational stage-specific changes in mRNA and protein of CYP2D and CYP2C enzymes during rat pregnancy (Dickmann et al., 2008), no significant differences were observed between gestational stages in CYP1A2 activity or expression. This finding again highlights the fact that the effects of pregnancy are specific for a given P450 enzyme, and the mechanisms that result in altered P450 expression and activity during pregnancy vary between isozymes.

Role of Estrogens and Progesterone in Decreased CYP1A2 Activity. CYP1A2 can metabolize endogenous estrogens that have been suggested as potential regulators of CYP1A2. CYP1A2 activity is lower in women than in men (Relling et al., 1992), and hormone replacement therapy (Laine et al., 1999) decreases CYP1A2 activity. Hence, the effect of 17β-estradiol and progesterone on CYP1A2 mRNA was tested in primary rat hepatocytes, and the effect of common sex steroids as reversible CYP1A2 inhibitors was measured.

In contrast to the clinical findings, estrogens or progesterone had no effect on CYP1A2 mRNA or activity at biologically relevant concentrations. 17β-estradiol (10, 100, or 1000 pM) and progesterone (10, 100, and 1000 nM) had no effect (p > 0.05) on CYP1A2 mRNA after either 24 or 48 h of treatment, except for a <15% decrease (p < 0.05) in CYP1A2 mRNA after a 1000 nM progesterone treatment for 24 h. In addition, treatment of the rat hepatocytes with estrogen or progesterone for up to 48 h did not change CYP1A2 activity measured by phenacetin O-deethylation (data not shown), suggesting that at physiological concentrations, these hormones do not inactivate CYP1A2, despite the report...
of increased CYP1A2 inhibition after preincubation with 300 μM progestosterone (Karjalainen et al., 2008). No inhibition of MROD was observed in CYP1A2 Supersomes at physiologically relevant concentrations of 17-β-estradiol, estrone, estradiol, and progesterone, and a maximum of 40% inhibition was observed at 20,000 nM concentration of these hormones (data not shown). This result is in agreement with previously determined Ki values of 17-β-estradiol (114 μM) and progesterone (710 μM) toward human CYP1A2 (Eugster et al., 1993; Karjalainen et al., 2008). Therefore, down-regulation or inhibition of CYP1A2 by increasing concentrations of these hormones during pregnancy is an unlikely reason for the observed decrease in CYP1A2-mediated clearance.

In conclusion, these data demonstrate that CYP1A2 protein expression is decreased during pregnancy, resulting in a decreased activity of CYP1A2. This decrease occurs in rat pregnancy and mimics the changes observed in human CYP1A2 activity during pregnancy supporting the use of the rat as a model to study mechanisms by which CYP1A2 changes during pregnancy. The decrease in CYP1A2 mRNA was smaller in magnitude than that observed for CYP1A2 protein and activity and was only observed during late pregnancy. This result suggests that mechanisms beyond regulation of CYP1A2 mRNA levels may be responsible for the early down-regulation of CYP1A2 protein and require further studies. The fact that changes in CYP1A2 activity in the rat during pregnancy closely mimic those observed in humans is important when the rat is used as a model species for studying developmental toxicity of CYP1A2 substrates.

Authorship Contributions

Participated in research design: Walker, Dickmann, and Isoherranen.
Performed data analysis: Walker, Dickmann, and Isoherranen.
Wrote or contributed to the writing of the manuscript: Walker, Dickmann, and Isoherranen.

Department of Pharmaceutics, University of Washington, Seattle, Washington

ALYSA A. WALKER  
LESLIE DICKMANN

NINA ISOHERRANEN

1 Current affiliation: Amgen, Seattle, Washington.

References


Address correspondence to: Dr. Nina Isoherranen, Department of Pharmaceutics, University of Washington, Box 357610, Seattle, WA 98195-7610. E-mail: nl2@u.washington.edu