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Changes in maternal liver Cyp2c and Cyp2d expression and activity during rat pregnancy

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ABSTRACT

During human pregnancy, CYP2C9, CYP2C19, and CYP2D6 activities are altered. The aim of the current study was to determine if this phenomenon can be replicated in the rat, and to evaluate the mechanisms that contribute to the changes in Cyp2c and Cyp2d activity during pregnancy. The intrinsic clearance of dextromethorphan *O*-demethylation, a measure of Cyp2d2 activity, was decreased 80% at both days 9 and 19 of gestation when compared to non-pregnant controls. The decreased intrinsic clearance was a result of both decreased V_{max} and increased K_m-values at both days of gestation. Quantitative RT-PCR revealed that transcripts of Cyp2d2 and Cyp2d4 were significantly decreased at day 19 of pregnancy ($p < 0.05$) when compared to day 9 and non-pregnant controls. The decrease in Cyp2d mRNA levels correlated with a decrease in several nuclear receptor mRNA levels (RARα, RXRα, HNF1 and HNF3β) but not with the mRNA levels of nuclear receptors usually associated with regulation of P450 enzymes (PXR, CAR and HNF4α). In contrast, Cyp2c12 and Cyp2c6 transcription and protein expression were not significantly altered during rat pregnancy although the intrinsic clearance of Cyp2c6 mediated diclofenac 4′-hydroxylation was increased 2-fold on day 19 of gestation when compared to non-pregnant controls. The increase in intrinsic clearance was due to a decrease in the K_m-value for 4′-hydroxydiclofenac formation. These data show that pregnancy significantly alters the expression and activity of drug metabolizing enzymes in an enzyme and gestational stage specific manner. These changes are likely to have toxicological and therapeutic implications.

1. Introduction

There is substantial evidence that pregnancy changes the disposition of many drugs and modifies the activity of drug metabolizing enzymes [1–3]. Increased clearance may lead to a lack of therapeutic effect or prolonged titration to achieve desired effect, while decreased clearance may lead to an increased occurrence of side-effects or toxicity due to elevated drug concentrations. Changes in drug clearance may also change the exposure of the

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Abbreviations: RT-PCR, real time PCR; RAR, retinoic acid receptor; RXR, retinoid X receptor; HNF, hepatic nuclear factor; PXR, pregnane X receptor; CAR, constitutive androstane receptor; PBS, phosphate buffered saline; TE, tris-EDTA.

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developing fetus to the potentially harmful parent drug or its metabolites.

CYP2C9, CYP2C19, and CYP2D6 are major drug metabolizing enzymes and together account for approximately 40% of all Phase I drug metabolism [4]. CYP2C9 plays a major role in the metabolism of phenytoin and anti-inflammatory drugs, whereas CYP2D6 is important in the metabolism of almost all antidepressants, including fluoxetine, paroxetine, tricyclic antidepressants and several of the newer antidepressants [5]. Changes in CYP2C9 and CYP2D6 activity are important in the treatment of pregnant women, since many of their substrates are commonly administered to pregnant women. Increases in enzyme activity could result in significantly decreased plasma concentrations of their substrates during pregnancy, leading to therapeutic failure.

The available clinical data, although limited, suggests that CYP2C9 and CYP2D6 mediated metabolism is increased during pregnancy, while metabolism catalyzed by CYP2C19 is decreased. An increased clearance of phenytoin, an antiepileptic drug predominantly metabolized by CYP2C9, was reported during pregnancy in two studies [6,7] and phenytoin dosage had to be increased in 85% of pregnancies to maintain therapeutic efficacy [8]. In contrast, CYP2C19 mediated metabolism of proguanil to its active metabolite, cycloguanil, appears to decrease during pregnancy [9]. In a study of 44 women, the proguanil to cycloguanil plasma concentration ratio was 63% higher during the second and third trimesters of pregnancy compared to postpartum [9].

Two classical CYP2D6 probe substrates, dextromethorphan and metoprolol, have been used to assess CYP2D6 activity during human pregnancy. The data suggests that CYP2D6 activity increases significantly during pregnancy [3,10,11]. When evaluated at 26–30 weeks of gestation, the oral clearance of metoprolol increased 6-fold and the bioavailability decreased to half when compared to postpartum [10]. A significant decrease in the urinary dextromethorphan to dextrorphan metabolic ratio, indicative of an increase in CYP2D6 activity, was also observed at all trimesters of pregnancy [3]. Finally, in individuals genotyped for CYP2D6, a 53% decrease in dextromethorphan to dextrorphan plasma ratio was observed in extensive metabolizers whereas a 63% increase in the same ratio was observed in poor metabolizers during pregnancy [11].

The mechanisms underlying the indicated changes in CYP2C9, CYP2C19, and CYP2D6-mediated clearance during pregnancy are poorly understood. For this reason, the goal of the current study was to investigate changes in hepatic Cyp2c and Cyp2d expression and activity during pregnancy using the rat as a model and to investigate potential mechanisms behind any observed changes in Cyp2c or Cyp2d expression and activity. Hepatic Cyp2c and Cyp2d transcription and expression were measured by quantifying the mRNA and protein levels, as well as enzyme activity using isoform-specific probe substrates. Messenger RNA levels of nuclear hormone receptors known to regulate P450 expression were also measured and correlated to changes in P450 mRNA levels.

2. Materials and methods

2.1. Animal studies

Animals were cared for in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication [12]. The experimental studies were approved by the Institutional Animal Care and Use Committee at the University of Washington. Virgin female Sprague–Dawley rats 8 weeks of age were mated and pregnancy was determined by the detection of a vaginal plug. Males were then removed and the females divided in random order into two groups. The rats in group one ($n = 6$) were sacrificed at day 9 of gestation and the rats in group 2 ($n = 10$) on day 19 of gestation. An additional 6 virgin, age-matched females, from the same cohort of animals, were sacrificed as non-pregnant controls at the same days as the pregnant animals were sacrificed. The animals were sacrificed under deep isoflurane inhalation anesthesia by cardiac puncture and exsanguination between 9 a.m. and 12 noon. Blood was collected and the plasma harvested and stored at −80°C. Upon sacrifice, liver was perfused via the portal vein using ice cold PBS and liver, intestine, kidney, and brain tissues were harvested, weighed, and washed in ice-cold PBS. The tissue samples were snap frozen in liquid nitrogen and stored at −80°C until further processing.

2.2. Preparation of rat liver microsomes

All procedures were performed on ice. Approximately 5 g of rat liver tissue was minced in 10 mL of homogenization buffer (50 mM potassium phosphate, pH 7.4, 0.25 M sucrose) and then homogenized in a glass tissue grinding tube by passing a tight-fitting Teflon pestle through the solution using an electric drill. The samples were then diluted to 100 mL final volume with homogenization buffer and centrifuged at 8,000 × *g* for 30 min at 4 °C. The supernatant was carefully poured off and centrifuged at 110,000 × *g* for 70 min at 4 °C. The supernatant was discarded and the pellet was resuspended in wash buffer (10 mM potassium phosphate, pH 7.4, 0.1 M potassium chloride) by vortexing until the microsomal pellet (and not the clear glycogen pellet) dislodged from the side of the tube. The pellet was then transferred to a glass tissue grinding tube and homogenized by hand using a Teflon pestle and resuspended in a final volume of 100 mL wash buffer. Samples were centrifuged again at 110,000 × *g* for 70 min at 4 °C, the supernatant was discarded and the pellet was resuspended in ~2 mL of storage buffer (50 mM potassium phosphate, pH 7.4, 0.25 M sucrose, 10 mM EDTA) by vortexing and then homogenizing by hand in a small glass tissue grinding tube with a tight-fitting pestle. The resulting rat liver microsome samples were aliquoted and stored at −80 °C until further use. Pooled microsomes from non-pregnant rats and from rats of gestational days 9 and 19 were prepared by combining equal amounts of microsomal protein from each liver in the group.

2.3. Spectral characterization of rat liver microsomes

Spectral studies were performed using a Varian Cary 3E UV–visible Spectrophotometer. Spectral P450 contents were