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Research Article

Regulation of Drug Disposition Gene Expression in Pregnant Mice with Car Receptor Activation

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Abstract. More than half of pregnant women use prescription medications in order to maintain both maternal and fetal health. The constitutive androstane receptor (Car) critically affects the disposition of chemicals by regulating the transcription of genes encoding metabolic enzymes and transporters. However, the effects of Car activation on chemical disposition during pregnancy are unclear. This study aims to determine the degree to which pregnancy alters the expression of drug metabolizing enzymes and transporters in response to the pharmacological activation of Car. To test this, pregnant C57BL/6 mice were administered IP doses of vehicle, or a potent Car agonist, TCPOBOP, on gestation days 14, 15 and 16. Hepatic mRNA and protein expression of Car target genes (phase I, II and transporters) were quantified on gestation day 17. Pregnancy-related changes, such as induction of Cyp2b10, Ugt1a1 and Sult1a1 and repression of Ugt1a6, Gsta1, Gsta2 and Mrp6, were observed. Interestingly, the induction of Cyp2b10, Gsta1, Gsta2 and Mrp2–4 mRNAs by TCPOBOP was attenuated in maternal livers suggesting that Car activation is impeded by the biochemical and/or physiological changes that occur during gestation. Taken together, these findings suggest that pregnancy and pharmacological activation of Car can differentially regulate the expression of drug metabolism and transport genes.

Keywords: Car; transporters; metabolism; pregnancy; nuclear receptor

1. Introduction

Pharmacotherapy is necessary to treat new and pre-existing conditions during pregnancy. It is estimated that 64% of women use prescription drugs (excluding vitamins and minerals) during pregnancy [1]. There are a number of adaptations in the mother that occur to support the needs of the growing fetus [2]. The maternal liver undergoes significant physiological and biochemical changes that, in turn, alter the disposition and metabolism of endobiotics such as lipids, bile acids, glucose and cholesterol, as well as xenobiotics [2–5]. Moreover, the expansion of plasma volume during pregnancy contributes to elevations in cardiac output and increased hepatic blood flow, which can enhance the clearance of high extraction ratio drugs [4]. Despite the need for pharmacotherapy, little data exist regarding

the safety and appropriate dosing regimens of drugs due to ethical concerns that preclude the participation of pregnant women in randomized clinical trials.

The constitutive androstane receptor (Human CAR/rodent Car, *NR1I3*) is a nuclear receptor that is highly enriched in the liver [6]. As its name suggests, in either the absence, but particularly in the presence of an agonist, Car heterodimerizes with the retinoid X receptor alpha and binds to cognate response elements of target genes, thereby up-regulating their transcription [reviewed in 7]. Chemical activators of Car are structurally diverse and include drugs like phenobarbital, the fungicide propiconazole, as well as synthetic compounds such as 1,4-bis(2-(3,5-dichloropyridyloxy)) benzene (TCPOBOP) [8].

CAR/Car is considered a master regulator of hepatic drug disposition because downstream targets include a number of drug metabolizing enzymes and xenobiotic transporters. Activation of CAR/Car in the liver is routinely monitored by the up-regulation of the human cytochrome P450 enzyme, CYP2B6, or the mouse ortholog Cyp2b10 [9, 10]. In addition, rodent Car has been shown to regulate several phase II conjugating enzymes, including members of the UDP-glucuronosyltransferase (Ugt), sulfotransferase (Sult) and glutathione *S*-transferase (Gst) families [7, 11–13]. Ligand-activated Car has also been shown to alter the expression of hepatic uptake transporters including the organic anion transporter proteins (Oatps) [14, 15] and efflux transporters such as the multidrug resistance-associated proteins (Mrps) in rodents [6, 16].

Our laboratory, along with others, has previously demonstrated that the expression of nuclear receptors and their prototypical target genes are repressed in mice during pregnancy [17–19]. The altered function of transcription factors in the maternal liver corresponds with variable changes in the expression and activity of phase I and phase II enzymes, and an overall down-regulation of transporters. Notably, we have observed that pregnant mice have reduced levels of hepatic Car target genes including Ugt1a6, Gsta1, and Mrp3 [17, 19]. Early studies revealed the ability of prototypical microsomal enzyme inducers such as phenobarbital to alter xenobiotic metabolism during pregnancy [20, 21]. Treatment of pregnant guinea pigs with phenobarbital resulted in enhanced total Cyp content [22]. Likewise, administration of phenobarbital induced the glucuronidation of estrone and estradiol to a greater extent in pregnant rats than in non-pregnant females [21]. However, these studies preceded our knowledge of individual Cyp and Ugt isoforms that are differentially up- or down-regulated by phenobarbital. As a result, further investigation is needed in the regulation of Car targets during pregnancy using a specific and potent pharmacological agonist.

It is recognized that pregnancy differs between species with respect to gestation length, number of offspring, placentation, and other factors [reviewed in 23]. Nonetheless, rodents are a useful model to assess adaptive responses to pregnancy and are routinely used in reproductive toxicology studies to assess the safety of new pharmaceuticals. Therefore, the purpose of this study was to determine to what degree pregnancy alters the expression of drug metabolizing enzymes and transporters in response to the pharmacological activation of Car in mice.

2. Materials and Methods

2.1. Chemicals. All chemicals including 1,4-bis(2-(3,5-dichloropyridyloxy)) benzene (TCPOBOP) were obtained from Sigma Aldrich (St. Louis, MO), unless otherwise specified.

2.2. Animals. Adult male and female wild-type C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA). Mice were allowed access to feed and water *ad libitum*. All mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal care facility in temperature-, light-, and humidity-controlled rooms. The Rutgers University Institutional Animal Care and Use Committee approved all animal studies. A subset of female mice were mated overnight with male mice and separated the following morning (designated gestation day 0). The remaining female mice were utilized as virgin controls. Virgin and pregnant time-matched mice ($n = 6-9$) received an intraperitoneal injection of vehicle (5 ml/kg corn oil) or TCPOBOP (1 mg/kg) on gestation days 14, 15 and 16. Livers were collected, weighed, and snap frozen on gestation day 17.

2.3. RNA Isolation. Total RNA was isolated from snap frozen whole livers using RNABee (Tel-Test, Friendswood, TX) and the RNeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer recommendations. Concentrations of total RNA were quantified by a NanoDrop spectrophotometer (Fisher Scientific, Pittsburgh, PA). Complimentary DNA (cDNA) was synthesized using High Capacity cDNA Synthesis for quantitative PCR (Applied Biosystems, Foster City, CA).

2.4. Quantitative PCR. Quantitative PCR was performed in 384-well plate format using the Viia7 Real Time PCR machine (Applied Biosystems, Warrington, UK). Specific forward and reverse primers are listed in Table 1. SYBR Green fluorescence (Applied Biosystems) was used to detect amplified products. Ribosomal protein l13a (Rpl13a) was used as a reference gene. Ct values were converted to delta delta Ct values by comparing to Rpl13a.

2.5. Western Blot Analysis. Liver tissue was homogenized in 10 mM Tris base and 150 mM sucrose buffer, pH 7.5

Table 1: qPCR Primer Sequences.

Primer	Forward (5' to 3')	Reverse (5' to 3')
Car	ATCTGCCGCTCTTCCGGTCC	GGAACCCTGCATGGACTGCGT
Cyp2b10	TGCTGTCGTTGAGCCAACCTTCA	GGGGCTCCCTGGGATTTCCG
Ugt1a1	TGTCTTTCAACTCAGACCGC	AGATGCAGGGCTCAGAAGAT
Ugt1a6	AATTCAGATGCTGGCTGATG	AAGTGTCTGAGCAGCAGGAA
Ugt1a9	ACTGCCTCCAGAAGAAGTCA	CACAGGACCGTCTGAGGAA
Ugt2b5	GGCCAACCACCTTAGTTGAG	TTCCATATCCTTAGGCAAGGGTT
Gsta1	CCCCTTTCCCTCTGCTGAAG	TGCAGCTTCACTGAATCTTGAAAGC
Gsta2	CACACTCCTCTGGAGCTGGAT	CCCGGGCATTGAAGTAGTGA
Sult1a1	CCCGTCTATGCCCGGATAC	GGGCTGGTGTCTCTTTCAGAGT
Oatp1a1	CCAACGCAAGATCCAACAGAGTGTG	TCGGGCCAACAATCTTCCCCAT
Oatp1a4	CGTGGGGATACCGAATTGTCT	GCTTTTCCAAGATCAAGGCATTT
Mrp2	AGCAGGTGTTCGTTGTGTGT	AGCCAAGTGCATAGGTAGAGAAT
Mrp3	CTGGGTCCCCTGCATCTAC	GCCGTCTTGAGCCTGGATAAC
Mrp4	ACCCTCGTTGAAAGAC	TGAAGCCGATTCTCCCTTC
Mrp6	TGTCTGCAAGCCATCGGACTGTTTG	TGGAAAAGCGGTTCAGCAGGTTCC
Rpl13a	CAAGAAAAAGCGGATGGTGG	TCCGTAACCTCAAGATCT

using the TissueLyser LT Adapter (Qiagen), as per the manufacturer's protocol. Protein concentrations were determined using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). Protein homogenates (50 μg) were separated on a 4–12% NUPAGE Novex Bis-Tris gel (Invitrogen, Carlsbad, CA) and transferred to a polyvinylidene fluoride membrane. Western blot detection of Ugt1a1, 1a9, and Mrp2–4 and 6 was performed as described previously [17, 24]. Protein bands were visualized using SuperSignal West Dura Chemiluminscent Substrate (Thermo Scientific, Pittsburgh, PA) on a FluorChemE Systems Imager (Protein Simple, Santa Clara, CA). Relative band intensities were semi-quantified utilizing Alpha View SA software (Protein Simple) and normalized to a loading control beta-actin (ab8227, Abcam, Cambridge, MA).

2.6. Indirect Immunofluorescence. Immunofluorescent staining was performed on liver cryosections (6 μm) as previously described [19]. Images were acquired on a Zeiss Observer D1 microscope at 200x magnification using a Jenoptik camera. All sections were stained and imaged under uniform conditions for each antibody. Negative controls without primary antibody were also included (data not shown).

2.7. Data Analysis. All results are expressed as mean $\pm$ standard error (SE). Data are normalized to virgin controls (set to 1.0). mRNA and protein expression were analyzed using a 2-way ANOVA or a 1-way ANOVA followed by a Newman-Keuls multiple comparison post-hoc test when appropriate using GraphPad Prism V6 (GraphPad, La Jolla, CA) software. Significance was set at $P \leq 0.05$.

3. Results and Discussion

3.1. Activation of Car in pregnant mice following TCPOBOP administration. Pregnancy did not significantly alter Car mRNA levels, while treatment with TCPOBOP reduced its levels 45 to 50% in both virgin and pregnant mice as compared to virgin controls (Figure 1). A 50% repression of Car mRNA by TCPOBOP was similar to prior studies in non-pregnant animals [6, 7, 25]. The strong activation of Car by TCPOBOP may initiate a negative feedback repression of its own expression to limit further signaling.

The prototypical human CAR target gene, CYP2B6, contributes to the metabolism of up to 8% of commonly used drugs, including the pain reliever methadone and sertraline, an antidepressant often prescribed in pregnancy [5, 21, 26, 27]. The mRNA expression of Cyp2b10, the mouse ortholog of CYP2B6, was enhanced by nearly 8-fold in pregnant mice, and was further induced by TCPOBOP in virgin and pregnant mice by 22- and 18.5-fold, respectively (Figure 1). There are conflicting reports regarding the adaptive regulation of Cyp2b10 mRNA during pregnancy with different reports demonstrating down-regulation [2, 17], up-regulation [29], or no change in its expression [28]. Nonetheless, these data demonstrate that Car activation was achieved in mice treated with TCPOBOP. Likewise, enhanced liver-to-body

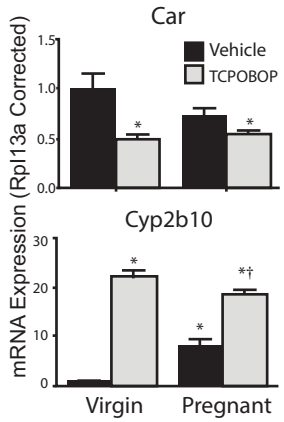

Figure 1: **Expression of Car and target gene Cyp2b10 in pregnant mice treated with TCPOBOP.** Hepatic mRNA expression of Car and its target gene Cyp2b10 was quantified in virgin and pregnant mice treated with vehicle or TCPOBOP. Data were normalized to vehicle-treated virgin controls (set to 1.0) and presented as mean relative expression $\pm$ SE ($n = 6-9$). Black bars represent vehicle-treated mice, and grey bars represent TCPOBOP-treated mice. Asterisks (*) represent differences ($P \leq 0.05$) compared with vehicle-treated virgin mice. Daggers (†) represent differences ($P \leq 0.05$) compared with vehicle-treated pregnant mice.

weight ratios were observed in both virgin and pregnant mice treated with TCPOBOP (see Supplemental Figure 1 in Supplementary Material available online at http://www.agialpress.com/journals/nurr/2016/101193/), confirming liver enlargement typically associated with Car activation in rodents [30].

3.2. Regulation of phase II metabolizing enzymes in pregnant mice following Car activation. Maternal livers exhibited differential changes in the expression of Ugt isoforms. Notably, pregnant mice had enhanced Ugt1a1 mRNA (35%), repressed Ugt1a6 mRNA (80%) and no change in levels of Ugt1a9 or 2b5 mRNAs compared to virgin controls (Figure 2(a)). Treatment with TCPOBOP enhanced expression of Ugt1a1 and 1a9 mRNAs in virgin as well as pregnant mice. Tolson et al. previously reported that activation of Car by phenobarbital or TCPOBOP increased the expression of Ugt1a1 and Ugt1a9, suggesting that Car may serve as a general regulator of glucuronidation enzymes [7]. While western blot confirmed that similar to mRNA levels, Ugt1a1 protein expression was increased by 27% in TCPOBOP-treated virgin mice, the same was not observed for Ugt1a9 (Figure 2(b)). However, both vehicle- and TCPOBOP-treated pregnant mice had a 15 to 20% reduction in Ugt1a1 protein levels compared to vehicle-treated virgin controls.

Compared to expression in vehicle-treated virgin controls, livers of virgin and pregnant mice treated with TCPOBOP showed reduced Ugt1a6 mRNAs. A similar down-regulation of Ugt1a6 was observed with TCPOBOP treatment in virgin and pregnant mice. The repression of Ugt1a6 mRNA by TCPOBOP was surprising given that Ugt1a6 has been shown to be up-regulated by prototypical enzyme inducers of Car in the livers of mice [12] and rats [31]. In a similar study, Ugt1a6 expression was unaltered in male mice treated with 300 μg/kg of TCPOBOP for four days [12]. Possible explanations for the divergent responses include variation in dose, and potential gender differences in the regulation of Ugt1a6.

Gsta1 and a2 are known targets of Car activation in mouse livers [11, 32]. Compared to vehicle-treated virgin mice, pregnancy decreased the mRNA expression of both Gsta1 and Gsta2 by 84 and 78%, respectively (Figure 3). TCPOBOP-mediated induction of both Gst isoforms was somewhat attenuated in pregnant mice compared to virgin mice. Consistent with a previous report, Sult1a1 mRNA was increased by 55 to 75% in pregnancy [17]; however administration of TCPOBOP did not further alter its expression.

3.3. Regulation of hepatic transporters in pregnant mice following Car activation. Pregnancy did not significantly change the expression of the uptake transporters Oatp1a1 or Oatp1a4, although opposing trends for enhanced Oatp1a1 and reduced Oatp1a4 levels were observed (Figure 4). Similar to a prior study in male mice, treatment with TCPOBOP reduced hepatic mRNA expression of Oatp1a1 [14]. While this trend was observed in both virgin and pregnant TCPOBOP-treated mice, Oatp1a1 was significantly repressed only in TCPOBOP-treated pregnant mice, as compared to vehicle-treated counterparts.

Our laboratory has previously demonstrated that Mrps, a group of efflux transporters, are differentially regulated in the maternal liver during pregnancy. Specifically, repression of Mrp2, 3 and 6 and induction of Mrp4 mRNAs were observed in pregnant mice [19]. In the present study, we observed similar pregnancy-related trends in vehicle-treated pregnant mice with the most notable change being the down-regulation of Mrp6 by 50% (Figure 5). As expected, TCPOBOP treatment in virgin mice up-regulated the mRNA expression of Mrp2 (0.76-fold), Mrp3 (0.68-fold), and Mrp4 (18-fold) [6, 16]. Likewise, TCPOBOP increased Mrp2–4 mRNAs in pregnant mice, though the magnitude of Mrp3 and 4 inductions were less than those observed in virgin mice. At the protein level, the up-regulation of Mrp2–4 proteins by TCPOBOP was also attenuated in pregnant mice compared to virgin mice (Figure 6). For example, Mrp2 protein was induced 2.6-fold in virgin mice treated with TCPOBOP, but only 1.6-fold in pregnant mice. It has been shown previously that reproductive hormones can mediate the internalization of transporters on the canalicular membrane of hepatocytes. Of importance to this study, Mrp2 localization can be disrupted by a metabolite of estradiol, estradiol-17β-D-glucuronide [33]. Indirect immunofluorescence confirmed the apical localization of Mrp2 to the canalicular membrane of hepatocytes (Figure 7). The intensity of staining of Mrp2 in liver sections was enhanced by TCPOBOP treatment in

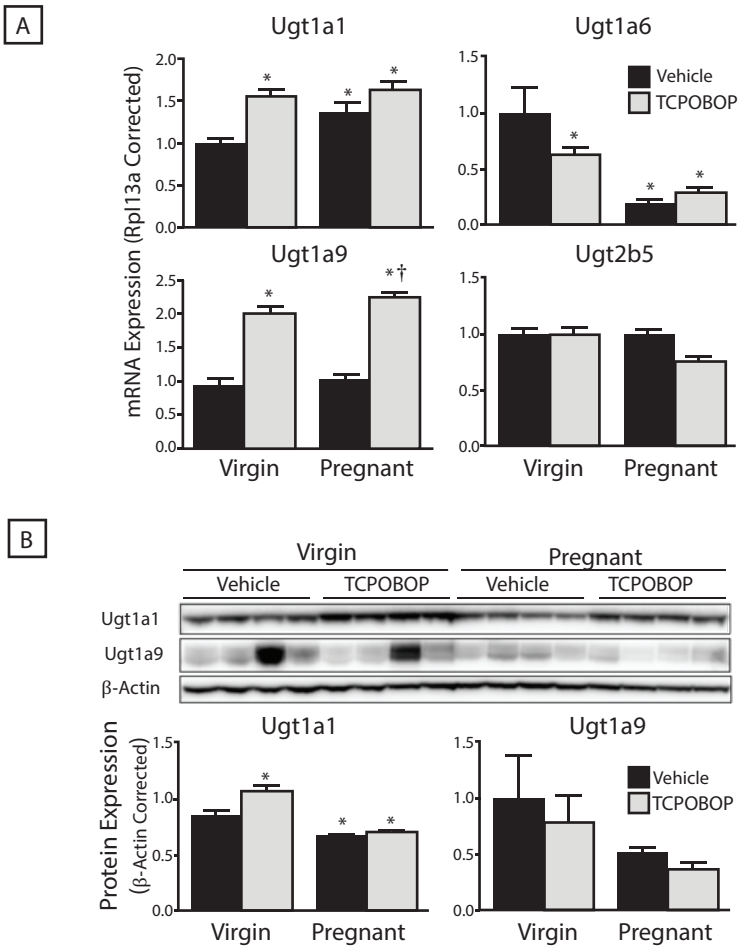

Figure 2: **Expression of Ugt enzymes in pregnant mice treated with TCPOBOP.** Hepatic (A) mRNA and (B) protein expression of Ugt enzymes were quantified in virgin and pregnant mice treated with vehicle or TCPOBOP. Representative western blots are shown and semi-quantification presented below. Data were normalized to virgin vehicle-treated controls (set to 1.0) and presented as mean relative expression $\pm$ SE ($n = 6 - 9$). Black bars represent vehicle-treated mice, and grey bars represent TCPOBOP-treated mice. Asterisks (*) represent differences ($P \leq 0.05$) compared with vehicle-treated virgin mice. Daggers (†) represent differences ($P \leq 0.05$) compared with vehicle-treated pregnant mice.

both virgin and pregnant mice; however, the overall intensity was lower in sections from pregnant mice.

Compared to Mrp2–4, the regulation of Mrp6 expression by Car is quite different. A prior study in male mice has demonstrated the ability of Car activation by phenobarbital and TCPOBOP to induce Mrp6 mRNAs [16]. Interestingly, we observed the opposite in both virgin and pregnant mice treated with TCPOBOP. Similar to the down-regulation of Mrp6 observed in vehicle-treated pregnant mice, the administration of TCPOBOP also led to reduced Mrp6 mRNA expression by 45 and 58% in virgin and pregnant mice, respectively (Figure 5). Consistent with the down-regulation of mRNA expression in pregnant mice, hepatic Mrp6 protein expression was reduced by 35–50%. Both virgin and pregnant TCPOBOP-treated mice showed comparable down-regulation of mRNA and protein expression (Figure 6). Indirect immunofluorescence revealed localization of Mrp6 to the sinusoidal membrane of hepatocytes that was uniform throughout the lobule (Figure 7). Compared to vehicle-treated virgin controls, the intensity of staining was lower in livers from TCPOBOP-treated virgin mice and even further reduced in both vehicle- and TCPOBOP-treated pregnant mice. The mechanism(s) underlying the down-regulation of Mrp6 during pregnancy and lack of response to TCPOBOP is unclear. In comparison to other hepatic Mrp transporters, Mrp6 has fewer identified substrates [34–36]. Additionally, whereas Mrp2–5 were shown to be induced in altered physiological states, such as models of extrahepatic cholestasis and type I diabetes, Mrp6 was unchanged in mice [37, 38]. Therefore, it is likely that Mrp6 may not be an important compensatory pathway for basolateral excretion of endo- and xenobiotics, unlike its counterparts Mrp3 and Mrp4.

In conclusion, the current study assessed the responsiveness of the mouse liver to Car activation by the specific

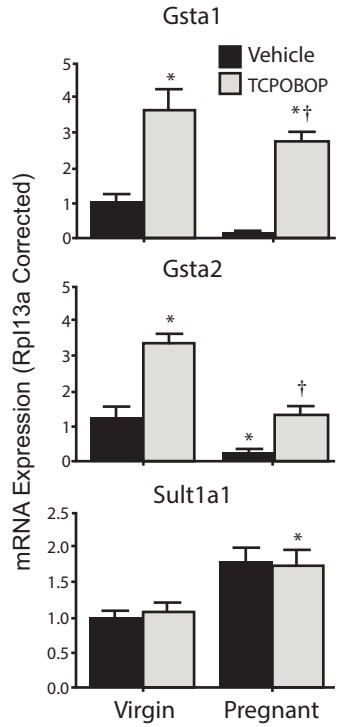

Figure 3: **Expression of Gst and Sult enzymes in pregnant mice treated with TCPOBOP.** Hepatic mRNA expression of phase II metabolic enzymes was quantified in virgin and pregnant mice treated with vehicle or TCPOBOP. Data were normalized to vehicle-treated virgin controls (set to 1.0) and presented as mean relative expression $\pm$ SE ($n = 6 - 9$). Black bars represent vehicle-treated mice, and grey bars represent TCPOBOP-treated mice. Asterisks (*) represent differences ($P \leq 0.05$) compared with vehicle-treated virgin mice. Daggers (†) represent differences ($P \leq 0.05$) compared with vehicle-treated pregnant mice.

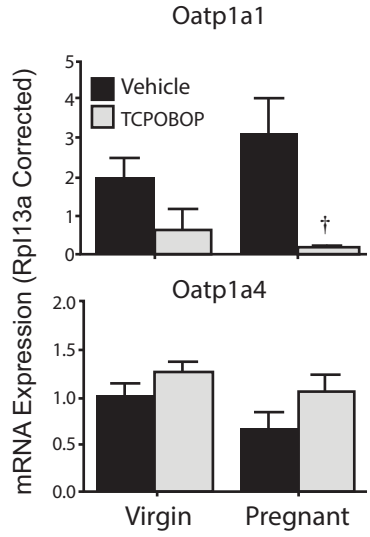

Figure 4: **Expression of uptake transporter genes in pregnant mice treated with TCPOBOP.** Hepatic mRNA expression of uptake transporters Oatp1a1 and Oatp1a4 was quantified in virgin and pregnant mice treated with vehicle or TCPOBOP. Data were normalized to vehicle-treated virgin controls (set to 1.0) and presented as mean relative expression $\pm$ SE ($n = 6 - 9$). Black bars represent vehicle-treated mice, and grey bars represent TCPOBOP-treated mice. Daggers (†) represent differences ($P \leq 0.05$) compared with vehicle-treated pregnant mice.

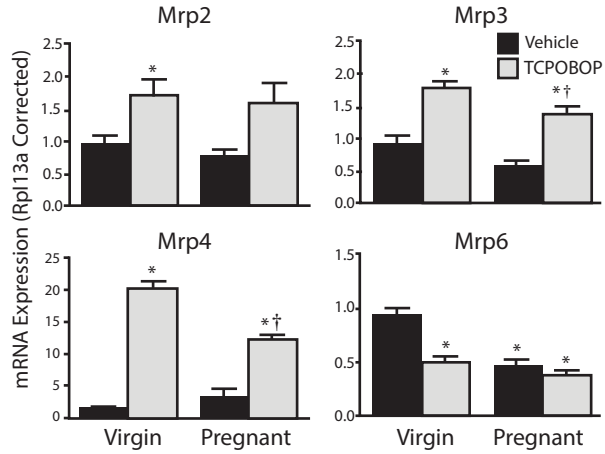

Figure 5: **Expression of efflux transporter genes in pregnant mice treated with TCPOBOP.** Hepatic mRNA expression of efflux transporters was quantified in virgin and pregnant mice treated with vehicle or TCPOBOP. Data were normalized to vehicle-treated virgin controls (set to 1.0) and presented as mean relative expression ± SE ($n = 6 - 9$). Black bars represent vehicle-treated mice, and grey bars represent TCPOBOP-treated mice. Asterisks (*) represent differences ($P \leq 0.05$) compared with vehicle-treated virgin mice. Daggers (†) represent differences ($P \leq 0.05$) compared with vehicle-treated pregnant mice.

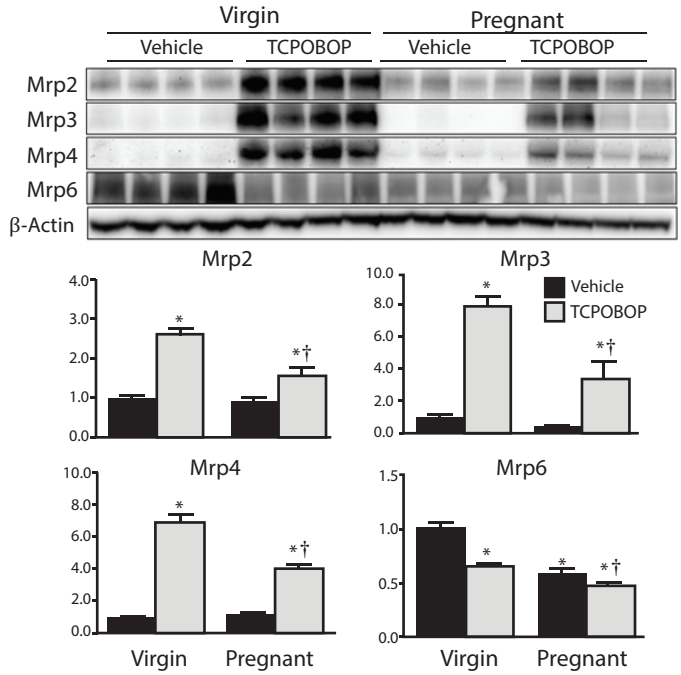

Figure 6: **Expression of efflux transporter proteins in pregnant mice treated with TCPOBOP.** Protein expression was quantified by western blot analysis using whole liver homogenates from virgin and pregnant mice treated with vehicle or TCPOBOP. Representative western blots are shown and semi-quantification presented below. Data were normalized to virgin vehicle-treated controls (set to 1.0) and presented as mean relative expression ± SE ($n = 6 - 9$). Black bars represent vehicle-treated mice, and grey bars represent TCPOBOP-treated mice. Asterisks (*) represent differences ($P \leq 0.05$) compared with vehicle-treated virgin mice. Daggers (†) represent differences ($P \leq 0.05$) compared with vehicle-treated pregnant mice.

and potent agonist, TCPOBOP, during pregnancy. Interestingly, the ability of TCPOBOP to induce known Car targets including Cyp2b10, Gsta1, Gsta2 and Mrp2–4 was impaired during pregnancy because for each of these genes, the maximal induction following TCPOBOP was lower in pregnant mice compared to virgin mice. These findings suggest that pregnancy impedes activation of Car in maternal mouse livers. Prior work showed that induction of microsomal estrone and estradiol glucuronyltransferase activity with phenobarbital was similar between non-pregnant and pregnant rabbits [21]. Although when adjusted for total liver size, greater microsomal activity was observed in

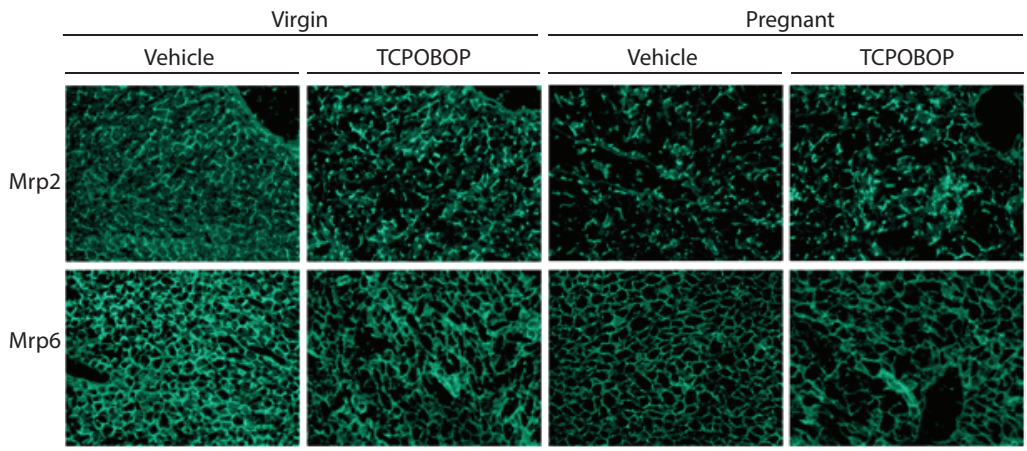

Figure 7: **Indirect immunofluorescent staining of Mrp2 and Mrp6 in the livers of pregnant mice treated with TCPOBOP.** Indirect immunofluorescence against canalicular transporter Mrp2 (*green*) and basolateral transporter Mrp6 (*green*) was conducted on liver cryosections (6 μm) obtained from virgin and pregnant mice treated with vehicle or TCPOBOP on gestation day 17. Representative images are shown. Magnification 200x.

livers from pregnant rabbits treated with phenobarbital compared to non-pregnant controls [21]. In our current study, Ugt1a1 and 1a9 proteins were not enhanced in pregnant mice treated with TCPOBOP, which may reflect differences in species, experimental endpoint and/or selection of Car activator. It is not clear whether the differences in Car responsiveness during pregnancy are due to changes in the pharmacokinetics of TCPOBOP or result from altered responsiveness of Car receptor activation. The induction of Cyp2b10 mRNA in vehicle-treated pregnant mice was not anticipated given the overall reduced signaling of Car-related targets and a trend for reduced Car mRNA itself (Figure 1). It should be noted that the translocation of Car protein to the nucleus was not quantified which may reveal additional insight into signaling pathways activated by pregnancy.

Future studies will address the ability of pregnancy-related hormones, such as estradiol, glucocorticoids, growth hormones, and progesterone, to influence Car function. Circulating concentrations of 17β-estradiol steadily increase from gestation days 11 to 17 in pregnant mice [19]. Koh et al. have demonstrated that pregnancy-relevant concentrations of estradiol ($>$100 nM) can activate CAR and estrogen receptor/activator protein-1 leading to the transactivation of the CYP2B6 gene in rat and human hepatocytes [39]. Moreover, the ability of exogenous estrogens (17β-estradiol and estrone) to modulate mouse Car function through the NR1 enhancer region of the *Cyp2b* promoter has been demonstrated *in vitro* using primary hepatocytes and in vivo with estradiol-treated mice [40]. Taken together, these data support the up-regulation of Cyp2b10 mRNA in vehicle-treated pregnant mice. In addition, further work is necessary to determine whether a similar attenuated response to CAR activation is observed in human livers during pregnancy. Despite the reduced signaling observed in maternal mouse livers, these findings suggest that the altered expression profiles of certain Car target genes as a result of pregnancy can be partially reversed by pharmacological intervention.

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Competing Interests

The authors declare that they have no competing interests.

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